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Contents

Über die Synthese einiger Methoxymethyl-alkyl-ketone und ihre Beziehung zum Aroma der Gärungsprodukte.

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Mit der Absicht, das Aroma verschiedener Gärungsprodukte zu studieren, hat der Verfasser vor einigen Jahren in Gemeinschaft mit T. HIGASI zuerst das *Acetoin* od. *Butan-2-ol3-on* in optisch inaktiver Form rein dargestellt und den Geruch genau durchprobiert(1). Das *Acetoin* ist bekanntlich in verschiedenen Gärungsprodukten enthalten. Neulich ist es auch im japanischen „*Saké*" (Reiswein) nachgewiesen worden. Es hat eigentümlichen, angenehmen Geruch, der dem Aroma des *Sakés* ähnelt. Es wurde von mehreren geübten *Saké*-Kennern im hiesigen Institut sorgfaltig untersucht. Nach der Beurteilung dieser Fachleute scheint es jedoch nicht die wichtigste Rolle bei der Qualität des *Sakés* zu spielen, obgleich es Nebenbestandteil seines Aromas bildet.

In Fortsetzung dieser Untersuchung hat der Verfasser neuerdings 4 neue Homologe des *Acetoin-methyläthers* von folgenden Formeln in reinem Zustande dargestellt:

I. $CH_3OCH_2\text{-}CO\text{-}C_2H_5$
Methoxymethyl-äthyl-keton (od. *1-Methoxy-butan-2-on*)

II. $CH_3OCH_2\text{-}CO\text{-}C_3H_7$ (*n*)
Methoxymethyl-n-propyl-keton (od. *1-Methoxy-pentan-2-on*)

III. $CH_3OCH_2\text{-}CO\text{-}C_4H_9$ (*n*)
Methoxymethyl-n-butyl-keton (od. *1-Methoxy-n-hexan-2-on*)

IV. $CH_3OCH_2\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}CH\ (CH_3)_2$
Methoxymethyl-isoamyl-keton (od. *1-Methoxy-5-methyl-hexan-2-on*)

Die Darstellung erfolgte wesentlich nach der Methode von D. Gauthier(2), die er zur Synthese der analogen Äthoxy-derivate verwendet hat, und zwar nach folgendem Schema:

$$CH_3OH + CH_2O + HCl \longrightarrow CH_3OCH_2Cl \xrightarrow{CuCN} CH_3OCH_2CN$$
$$\xrightarrow{RMgBr} CH_3OCH_2C\,(:NMgBr)\,R \xrightarrow{H_2O} CH_3OCH_2\text{-}CO\text{-}R$$

Ferner hat der Verfasser zur Charakterisierung der oben enwahnten Ketoläther die *Semicarbazone* und 2, 4-*Dinitrophenylhydrazone* hergestellt. Die Formeln, Farbe und Schmelzpunkte derselben werden im folgenden zusammengestellt:

Formel CH_3OCH_2-CO-R	Semicarbazon CH_3OCH_2-C(=N-NHCO-NH_2)-R Schmelzp.	2, 4-Dinitrophenylhydrazon CH_3OCH_2-C[=N-NH-$C_6H_3(NO_2)_2$]R Schmelzp.	Farbe
I. $CH_3OCH_2COC_2H_5$	84～85°	193～198.5°	Orange
II. CH_3OCH_2-CO-C_3H_7	97～98°	128.5～129°	〃
III. CH_3OCH_2-CO-C_4H_9 (n)	95°	93°	Seidenglänzend Hellgelb
IV. CH_3OCH_2-CO-C_5H_{11} (iso)	106～106°	109.5°	Gelb

Es sei hier bemerkt, dass Semicarbazone, wie die entsprechenden ursprunglichen Ketolather, mehr oder weniger leicht loslich in Wasser sind und dass besonders das erste (I) so leicht loslich ist, dass es sich aus der wassrigen Losung nicht abscheidet, sondern erst durch Sattigen mit Natrium-acetat abgeschieden wird, was bereits von M. Sommelet(3) in einem analogen Falle berichtet wurde.

Die Gerüche der oben erwahnten Ketolather wurden in verschiedener Weise probiert, z. B. 1) als solche ohne Verdünnung, 2) als 5～10% ige Losung in 15% Alkohol, oder 3) in kleinsten Mengen zu gebrautem oder synthetischem *Saké* zugesetzt. Der letztere ist seit einigen Jahren im hiesigen Institut technisch dargestellt worden. In der Weise wurd festgestellt, dass diese Ketolather, wie es bei den meisten Riechstoffen der Fall ist, in reinem Zustande nicht angenehm riechen. Erst sehr verdünnt, entfalten sie den ihnen eigentumlichen Duft. Sie zeigen aber weder so kraftigen, lebendigen Geruch, wie z. B. Terpinylacetat, noch so süssen Duft, wie Blütenextrakt. Sie ahneln vielmehr dem Frucht- als dem Blütenaroma. Unter 4 Ketolathern riechen I und II in 0.1% iger Losung in 15% Alkohol anfangs atherartig, spater mehr esterartig, etwas an Acetoin und Acetol erinnernd. III und IV riechen in der oben angegebenen Konzentration zu stark und etwas widrig. In noch verdünnterer Losung werden sie angenehm, trotzdem sie einen butterartigen Geruch mit etwas schimmelartigem Beitone entfalten. Der Ketolather (IV) riecht ahnlich wie Cyclohexyl-acetat. In wasserverdünntem Alkohol in geringen Mengen zugesetzt, gibt er einen angenehmen Geruch, doch scheint es nicht das charakteristische Aroma des *Sakés* zu sein.

Der Verfasser beabsichtigt weiter noch höhere Glieder der Ketolather herzustellen, um deren Aroma zu studieren.

Experimenteller Teil

Monochlor-methylather: wurde nach der in „Organic Synthesis Bd. IX, S. 58" ausführlich angegebenen Vorschrift dargestellt. Als Trocknungsmittel wurd anstatt Chlorcalcium Phosphorpentoxyd verwendet. Um die ganze Operation mit der angegebenen Menge der Ausgangsmaterialien in der erwahnten Zeit auszuführen, sollte man moglichst rasch in reichlicher menge trockenes Salzsauregas in die Reaktionsmischung einleiten.

Methoxy-acetonitril: In einem mit gut wirkendem Rückflusskühler versehenen 1/2 *l*-Rundkolben, der am obersten Ende des Kuhlers ein $CaCl_2$-Rohr zum Abschluss der Feuchtigkeit besitzt, werden 102 g Kupfercyanür, die bei 120° zur Gewichtskonstante getrocknet und fein gepulvert worden sind, mit 92 g Monochlor-methylather unter Umschütteln gut benetzt. Bisweilen fangt die Reaktion nach etwa 10 Minuten unter gelinder Selbsterwarmung an. Wenn es aber nicht der Fall ist, muss man den Kolben vorsichtig in warmes Wasser von etwa 50° eintauchen, bis die Reaktion eben einsetzt. Man hüte sich dabei, dass die Reaktion nicht zu weit geht. Sonst findet manchmal unkontrollierbares Selbstaufkochen statt, und die Ausbeute wird dementsprechend schlechter. Am zweckmassigsten schüttelt man den Kolben und lasst etwa 20 Minuten bei Zimmertemperatur stehen, bis die erste Reaktion vollig zum Stillstand kommt. Dann erwarmt man sorgfaltig im Wasserbade von 50 ～56°. Die Reaktion beginnt wieder von Rand des Kolbens. Es wird durch abwechselnde Erwarmung und Kühlung reguliert, bis sich keine Blasen mehr entwickeln und der Inhalt des Kolbens zu steinharten Kuchen erstarrt, bei dem es sich vermutlich um ein Additionsprodukt des Kupfercyanürs handelt.

Nun erhitzt man nach Belieben den Kolben in kochendem Wasserbade. Die harte Masse wird dadurch allmahlich unter Abscheidung des Kupferchlorürs dünnflüssig und verwandelt sich schliesslich in eine dunkelbraune Flüssigkeit. Der ganze Prozess nimmt bis dahin etwa 45 Minuten in Anspruch. Um die Reaktion zu vervollstandigen, kocht man weiter 20 Minuten im Olbade, bringt die Flüssigkeit, wahrned sie noch heisst ist, in einen einfachen Destillierkolben von etwa 20 ccm Inhalt, und destilliert unter Erhitzen in einem Olbade (Badtemperatur: anfangs 150°, zuletzt 210°) zuerst bei gewohnlichem, spater bei vermindertem Drucke (ca. 50 mm) so lange, bis kein Destillat mehr übergeht. Das vereinigte Destillat betragt 71 g. Es wird noch zweimal fraktionierter Destillation unterworfen. Die sehr geringe erste Fraktion enthalt das isomere Isonitril und riecht widrig. Die Hauptfraktion, die bei 110～122° (hauptsachlich bei 119°) übergeht, bildet reines Methoxy-acetonitril. Es ist wasserklare Flüssigkeit und riecht ahnlich wie Ethylacetat, aber angenehmer. In zugeschmolzenem Rohr aufbewahrt, bleibt es nach 5 Monaten noch unverandert. Ausbeute sehr gut.

METHOXYMETHYL-ALKYL-KETONE CH_3OCH_2-CO-R.

Vier Methoxymethyl-alkyl-ketone von der allgemeinen Formel CH_3OCH_2-COR wurden durch Umesetzung des Methoxy-acetonitrils mit den entsprechenden *Grignard*schen Reagenzien in wesentlich derselben Weise dargestellt. Im folgenden wird nur ein Beispiel von *Methoxymethyl-äthyl-keton* angegeben.

Methoxymethyl-äthyl-keton oder *1-Methoxy-butan-2-on* (*I*).

Wie bereits erwahnt, verlauft die Beaktion zwischen Methoxyacetonitril

und Alkyl-magnesium-bromid nach folgendem Schema:

$$CH_3OCH_2CN + C_2H_5MgBr \longrightarrow$$

$$CH_3OCH_2\text{-}C(=NMgBr)\text{-}C_2H_5 \xrightarrow{H_2O} CH_3OCH_2\text{-}CO\text{-}C_2H_5$$

Man bereitet zunächst in üblicher Weise in Wasserstoffatmosphere eine Lösung von Äthyl-Mg-bromid aus 17 g (0.7 Mol) Mg-Spänen, 76 g (0.7 Mol) Äthylbromid und etwa 230 ccm absolutem Äther und gibt dazu eine Lösung von 35 g (0.5 Mol) Methoxy-acetonitril im gleichen Volumen absoluten Äthers tropfenweise unter Kühlung mit Eiswasser zu. Die Reaktion tritt sehr lebhaft ein, und es scheidet sich ein weisser Niederschlag aus, der anfangs rasch verschwindet, spater aber ungelöst bleibt. Nach dem Zusatz der ganzen Menge Methoxy-acetonitril lasst man das Reaktionsgemisch über Nacht stehen. Dann wird es 30∼40 Minuten auf dem Wasserbade in gelindem Sieden erhalten, mit Kaltemischung gekühlt und mit Eiswasser versetzt. Es scheidet sich dadurch Magnesiumoxyd ab. Man gibt nun soviel kalte 20%ige Schwefelsaure zu, bis der abgeschiedene Niederschlag wieder klar gelöst ist und die Lösung schwach sauer reagiert. Nach kurzem Stehen wird die atherische Schicht von den wassrigen getrennt und der Äther auf dem Wasserbade abgedampft. Die dadurch zurückgebliebene Flüssigkeit wird mit der wassrigen Lösung vereinigt, und nach dem Zusatz von kleinen Mengen Natriumcarbonat destilliert man den Ketolather mit Wasserdampf ab, der sich teilweise als Öl abscheidet. Die Destillation wird so lange fortgesetzt, bis das Destillat beim Sattigen mit wasserfreiem Kaliumcarbonat kein Öl mehr abscheidet. Das vereingte Destillat wird nun mit Kaliumcarbonat gesattigt und der dadurch ausgeschiedene rohe Ketolather mit 40%iger wassriger Lösung von 52 g (0.5 Mol) reinem Natriumbisulfit gut gesechüttelt, dreimal mit Äther extrahiert, um Verunreinigungen zu entfernen, wieder mit einer gesattigten Kaliumcarbonatlösung bis zu deutlicher alkalischer Reaktion versetzt und in der oben erwähnten Weise mit Wasserdampf destilliert. Aus dem wassrigen Destillat scheidet sich nach dem Sattigen mit Kaliumcarbonat das Methoxymethyl-athyl-keton als Öl ab, das mit Chlorcalcium entwassert, sorgfaltig filtriert und schliesslich durch 2∼3 malige fraktionierte Destillation gereinigt wird. Die Ausbeute an reinem Produkt betragt 20 g. Siedepunkt etwa 130°.

Bei der Darstellung der oben erwahnten Ketolather seien noch folgende Bemerkungen gemacht.

1) Die Umsetzung des Methoxy-acetonitrils mit *Grignard*schen Reagenzien verlauft mit zunehmenden Kohlenstoffzahlen der letzteren immer langsamer. So muss man z. B. bei *Äthyl-magnesium-bromid* mit Eiswasser abkühlen, um die Reaktion zu ermassigen; bei *n-Butyl-magnesium-bromid* braucht man zu diesem Zwecke Wasser von etwa 15°, wahrend bei *Iso-Amyl-magnesium-chlorid* etwas höhere Temperatur nötig ist.

2) Bei der Zersetzung der Additionsprodukete des Methoxy-acetonitrils mit *Grignard*schen Reagenzien durch Zusatz von Eiswasser muss man mit

einem Glasstabe tüchtig umrühren, um das Berühren des Wassers mit den Additionsprodukten zu beschleunigen, da sonst die Umsetzung durch abgeschiedene Magnesiumoxyde stark gestört wird.

3) Um die Reaktionsprodukte von dem entstandenen Kupferchlorür möglichst rasch bei niederer Temperatur abzudestillieren, ist es am zweckmassigsten, einen kleinen Destillierkolben, der am untersten Teile des Halses mit einem Ableitungsrohr versehen ist, zu verwenden.

Semicarbazon des Methoxymethyl-äthyl-ketons: 1.5 g krystallisiertes Natriumacetat werden in 1.2 ccm Wasser heiss gelost, gekühlt und mit 1.2 g Semicarbazidhydrochlorid unter Umrühren versetzt, bis das letztere gelost ist. Gibt man nun 1 g Ketolather dazu, so tritt gelinde Selbsterwarmung ein. Nach etwa 3-stündigem Stehen bei Zimmertemperatur fügt man noch 2.5 g Natrium-acetat zu und lasst 24 Stunden stehen, bis das allmahlich sich abscheidende Semicarbazon nicht mehr zunimmt. Hierauf saugt man ab, und es wird sofort ohne Auswaschen uber Chlorcalcium getrocknet und zuerst aus Benzin (Siedep. 105 ∼110°), dann dreimal aus dem Gemisch von Benzol und Benzin (Siedep. 70 ∼90°) umkrystallisiert. In der Weise erhalt man das Semicarbazon als reine schneeweisse Blattchen, Schmelzp. 84∼85°.

Analyse:	3.274 mg Subst. gaben 5.425 mg CO_2 und 2.437 mg H_2O				
	4.065 mg Subst. gaben 0.921 ccm N_2 (19°, 754 mm)				
	$C_6H_{13}O_2N_3$ (159.1)	Ber.	C 45.25	H 8.23	N 26.39%
		Gef.	〃 45.19	〃 8.33	〃 26.27%

Es lost sich leicht in Alkohol, ziemlich leicht in Wasser, aber schwer in einer mit Natriumacetat gesattigten Losung.

2, 4-*Dinitrophenylhydrazon des Methoxymethyl-äthyl-ketons* wurde nach der allgemeinen, für wasserlosliche Carbonyl-verbindungen geeigneten Methode dargestellt: i. e. 1 g 2, 4-Dinitrophenylhydrazin wird mit 6 ccm 2 *N* Salzsaure, dann mit 5 ccm konz. Salzsaure gut verrührt und schliesslich mit 300 ccm 2 *N* Salzsaure versetzt. Das Gemisch wird lange Zeit gerührt, bis das anfangs gebildete schwerlösliche 2, 4-Dinitrophenylhydrazin-hydrochlorid nahezu vollstandig gelöst ist. Man filtriert nun von Spuren unlöslichen Rückstandes ab und gibt 0.5 g des Ketolathers unter Umrühren zu. Nach kurzer Zeit scheidet sich das Hydrazon als hellgelbe Krystalle ab, die nach einer halben Stunde abgesaugt, sukzessiv mit verdünnter Salzsaure, Wasser und Alkohol gewaschen und im Vakuum-Exsikkator über Chlorcalcium getrocknet werden. Ausbeute 1.3 g, Schmelzp. 193∼194°. Nach zweimaligem Umkrystallisieren aus Äthylacetat erhalt man reines 2, 4-Dinitrophenylhydrazon als orangegelbe Saulen, welche in heissem Alkohol schwer, in heissem Äthylacetat etwas leichter löslich sind. Ausbeute 1.1 g, Schmelzp. 198∼198.5°.

Analyse:	4.465 mg Subst. gaben 7.622 mg CO_2 und 1.988 mg H_2O				
	3.970 mg Subst. gaben 0.671 ccm N_2 (18.5°, 755 mm)				
	$C_{11}H_{14}O_5N_4$ (282.1)	Ber.	C 46.79	H 5.00	N 19.85%
		Gef.	〃 46.55	〃 4.98	〃 19.67%

Methoxymethyl-n-propyl-keton oder 1-Methoxy-pentan-2-on (II).

Es wurde aus 50 g *n*-Propylbromid, 9.7 g Magnesiumspänen, 20 g Methoxyacetonitril und Äther dargestellt. Es bildet farblose bewegliche Flüssigkeit. Ausbeute 13 g, Siedep. ca. 117°/175 mm.

Analyse:	3.575 mg Subst.	gaben 8.188 mg CO_2 und 3.358 mg H_2O		
	$C_6H_{12}O_2$	Ber.	C 62.03	H 10.34%
		Gef.	" 62.46	" 10.51%

Semicarbazon des Methoxymethyl-n-propyl-ketons: 4 g krystallisiertes Natriumacetat und 1.2 g Semicarbazid-hydrochlorid werded in 20 ccm Wasser gelöst, mit 1.0 g des Ketolathers und etwas Alkohol versetzt. Nach längerem Stehen wird der abgeschiedene Niederschlag abgenutscht. Der in Benzol leicht lösliche Teil wird aus einer Mischung von Bezol. und weing Petrolather wiederholt umkrystallisiert. So erhält man das reine Semicarbazon, das in Benzol und alkohol leicht, in Wasser ziemlich leicht löslich ist. Schmelzp. 97～98°. Es wurde über Chlorcalcium und Paraffin getrocknet und analysiert.

Analyse:	4.412 mg Subst.	gaben 7.857 mg CO_2 und 3.504 mg H_2O			
	4.488 mg Subst.	gaben 0.942 ccm N_2 (19°, 752.6 mm)			
	$C_7H_{15}O_2N_3$	Ber.	C 48.52	H 8.73	N 24.25%
		Gef.	" 48.57	" 8.89	" 24.30%

2, 4-*Dinitrophenylhydrazon des Methoxy-n-propyl-ketons*: 0.5 g 2, 4-Dinitrophenylhydrazin, 0.44 g (1.5 Mol) Ketolather und 15 ccm wasserfreier Alkohol werden in einem evakuierten geschlossenen Rohr zwei Stunden in kochendem Wasserbade erhitzt, die noch heisse klare Losung wird mit soviel Wasser versetzt, bis die Trübung beginnt, und stehen gelassen. Das Hydrazon scheidet sich dabei als hübsche lange Saulen ab, die abfiltriert, mit etwas Methylalkohol nachgewaschen, getrocknet und aus wasserfreiem Alkohol umkrystallisiert werden. 0.4 g lost sich in 10 ccm Alkohol; Schmelzp. 128.5～129°. Ausbeute sehr gut. Zur Analyse wurde es bei gewohnlicher Temperatur über Chlorcalcium getrocknet.

Analyse:	3.890 mg Subst.	gaben 6.975 mg CO_2 und 1.988 mg H_2O			
	4.671 mg Subst.	gaben 0.448 ccm N_2 (19°, 758.8 mm)			
	$C_{12}H_{16}O_6N_1$	Ber.	C 48.63	H 5.45	N 18.91%
		Gef.	" 48.90	" 5.72	" 18.61%

Methoxymethyl-n-butyl-keton oder 1-Methoxy-hexan-2-on (III).

Eine Lösung aus 95.9 g (0.7 Mol) *n*-Butylbromid, 17 g (0.7 Mol) Magnesium und 250 ccm Äther wird mit 39 g (0.55 Mol) Methoxyacetonitril in gleichem Volumen Äther versetzt. Die Reaktion ist zweckmassig durch Wasser von etwa 15° zu regulieren. Die Ausbeute an analysenreinem Produkte beträgt 23 g. Wasserklare, bewegliche Flüssigkeit. Siedep. 131～132° bei 173 mm. Nach 3-monatigem Aufbewahren in evakuiertem Rohr bleibt es fast ungefärbt.

Analyse: 4.434 mg Subst. gaben 10.539 mg CO_2 und 4.278 mg H_2O

4.033 mg Subst. gaben 9.623 mg CO_2 und 3.933 mg H_2O
$C_7H_{14}O_2$ Ber. C 64.61 H 10.77%
Gef. 〃 64.32; 65.11 〃 10.80; 10.91%

Semicarbazon des Methoxymethyl-n-butyl-ketons: Darstellung wie bei I aus 4 g krystallisiertem Natriumacetat, etwa 2 ccm Wasser und Semicarbazid-hydrochlorid und dazu 1.15 g Ketolather und wenig Alkohol. Der Reihe nach aus Benzol-Petroläther, Benzin vom Siedep. 80～100° und vom Siedep. 60～70° umkrystallisiert. Schneeweisse lange Saulen vom Schmelzp. 95°. Es löst sich ziemlich leicht in Wasser, leicht in Äther nnd Benzol, aber schwer in Petrolather vom Siedep. unter 60°, selbst bei Erwarmen, Um 0.6 g zu lösen braucht man etwa 50 c.c. Benzin. Beim Erkalten dieser Losung scheidet es sich zuerst als Ol ab, das doch bald zu Krystallmasse erstarrt. Nach dem Trocknen über Chlorcalcium-Paraffin wurd es analysiert.

Analyse: 3.013 mg Subst. gaben 5.670 mg CO_2 und 2.574 mg H_2O
3.752 mg Subst gaben 7.073 mg CO_2 und 3.063 mg H_2O
4.174 mg Snbst. gaben 0.797 ccm N_2 (17 5°, 758 8 mm)
$C_8H_1\ O_2N_3$ Ber C 51.30 H 9 16 N 22 44%
Gef. 〃 51.32; 51 41 〃 9 56; 9.13 〃 22.39%

2, 4-*Dinitrophenylhydrazon des Methoxymethyl n-butyl-ketons*: Darstellung wie bei II. Es krystallisiert aus Methylalkohol in hellgelben, seidenglanzenden, biegsamen und etwa 4 mm langen Nadeln. Ziemlich leicht loslich in heissem Methyl- und Äthylalkohol; Schmelzp. 93°.

Analyse: 3.462 mg Subst. gaben 6 440 mg CO_2 und 1 923 mg H_2O
3.398 mg Subst. gaben 6 238 mg CO_2 und 1 850 mg H_2O
4.096 mg Subst gaben 0 627 ccm N_2 (17.7°, 762 mm)
$C_{13}H_{18}O_6N_4$ Ber. C 50 29 H 5.85 N 18 10%
Gef. 〃 50 73; 50 07 〃 6 21; 6 09 〃 18.10%

Methoxymethyl-isoamyl-keton oder 1-Methoxy 5-methyl-hexan-2-on (IV).

Aus 42.4 g Isoamylchlorid, 9.7 g Magnesium, 22 g Methoxyacetonitril und 120 ccm Äther erhalt man 22 g. Siedep. 117° bei 87 mm; ca. 125° bei 110 mm. Farblose, bewegliche Flüssigkeit.

Analyse: 4.056 mg Subst. gaben 9 941 mg CO_2 und 4.098 mg H_2O
4.083 mg Subst gaben 10.106 mg CO_2 und 4 184 mg H_2O
$C_8H_{16}O_2$ Ber. C 66.66 H 11.11%
Gef. 〃 66.84; 67.50 〃 11.30; 11.46%

Semicarbazon des Methoxymethyl-isoamyl-ketons: Ausbeute an Rohprodukten betragt 1.27 g aus 1.4 g des Ketolathers, 1.2 g Semicarbazid-hydrochlorid und 1.5 g Natriumacetat. Nach einmaliger Umkrystallisation durch Auflosen in 7 ccm heissem absolutem Alkohol und nachtraglichen Zusatz von 35 ccm heissem Benzin wird es sofort rein. Schmelzp. 105～106°. Schneeweisse nadelformige Krystalle. In Wasser nicht so leicht löslich wie die niederen Homologe.

Zur Analyse wurde es über Chlorcalcium-Paraffin zur Gewichtskonstanz getrocknet.

Analyse: 4.323 mg Subst. gaben 8.484 mg CO_2 und 3.801 mg H_2O
4.229 mg Subst. gaben 0.748 ccm N_2 (17°, 758.8 mm)
$C_9H_{19}O_2N_3$ Ber. C 53.74 H 9.52 N 20.88%
Gef. 〃 53.52 〃 6.84 〃 20.76%

2, 4-*Dinitrophenylhydrazon des Methoxymethyl-n-isoamyl-ketons*: Darstellung aus 0.55 g des Ketoläthers, 0.69 g 2, 4-Dinitrophenylhydrazin und Alkohol; Ausbeute 1 g. Aus absolutem Alkohol umkrystallisiert, bildet es biegsame gelbe Nadeln vom Schmelzpunkt 109.5°. 0.5 g ist in etwa 10 g heissem Alkohol löslich.

Es wurde bei gewöhnlicher Temperatur über Chlorcalcium getrocknet und analysiert.

Analyse: 4.462 mg Subst. gaben 8.538 mg CO_2 und 2.609 mg H_2O
3.214 mg Subst. gaben 6.135 mg CO_2 und 1.899 mg H_2O
4.181 mg Subst. gaben 0.620 ccm N_2 (18.5°. 760.8 mm)
$C_{14}H_{20}O_6N_4$ Ber. C 41.83 H 6.22 N 17.28%
Gef. 〃 52.19; 52.06 〃 6.54; 6.61 〃 17.39%

Bei dieser Arbeit habe ich von Herrn Prof. Dr. U. Suzuki Anregung und Rat erhalten, wofür ich ihm auch hier meinen besten Dank ausspreche.

Literatur

(1) T. Higasi und S. Maruyama: *Bull. I. P. C. R.*, **7** (1929), 934~939 (Japanisch).
(2) D. Gautiher: *A. Ch.*, [8], **16** (1909), 289~358; siehe auch M. Sommelet: *Bull.*, [4], **1** (1907), 384~390.
(3) *loc. cit.*

Sterilising Action of Acids.

Second Report.—Sterilising action of saturated monobasic fatty acids. (1)

Sogo Tetsumoto.

(Received November 11, 1932.)

I studied on the sterilising action of mineral acids on microorganisms having the certain vital force and resisting power.

I reported on the Bul. of the Agr. Chem. S. of Japan[1] about the sterilising action of the same molecular concentration and pH, the effect of anions, undissociated molecules and that I made the comparative studies about the effect of sterilising power of each mineral acids. Following to above study, I

studied the sterilising action of saturated monobasic fatty acid ($C_nH_{2n+1}\cdot CO_2H$) on microorganisms to ascertain the effects of the number of C atom, pH, the same moleculer concentration, the chemical construction, anions, and undissociated molecules on the sterilising action.

Experiment.

(1) Experimental methods.

I took the same methods those I reported before on the Bul. of Agr. Chem. S. of Jap[1] already. So that now I record the name of used microorganisms and the resisting power for phenol aqueous solution for convenience.

Name of micoorganism		Staphyloccocus pyogenes aureus	Bac. typhosus	Proteus vulgaris, Hauser	Vib. Cholerae
Phenol aq. solution, times by weight		75	90	100	175
surviving	5	+	+	+	+
period.	10	+	±	±	±
(minut)	15	—	—	—	—

+ …… alive — …… perished ± …… sometimes perished or sometimes alive.

(2) Reagents.

I used saturated monobasic fatty acids $C_nH_{2n+1}\cdot CO_2H$: such as 13 acids.

Formic, Acetic, Propionic, Butyric, Isobutyric, Valeric, Isovaleric, Caproic, Isocaproic, Cenanthylic, Pelargonic, Capric.

Except Formic acid I used reagents manufactured by Kahlbaum Co. or Merck Co. and made lower dilute concentration than $N/10$.

(3) Concentration and pH of reagents.

Reagents are all weak organic dilute acid solutions and have no action such as buffer. So that pH of each acid must be found by next formulae.

$$pH = \frac{1}{2}\, p\, KHA - \frac{1}{2} \log C$$

KHA …… electric dissociation constant of acids.

p …… changed sign of logarithum of KHA.

C …… concentration of reagents …… normality.

As electric dissociation constants of Caprylic acid, Pelargonic acid and Capric acid are unknown, I determined pH of these acid by colorimetric method.

(4) Reagents, formulae, concentration and pH (calculated) are shown in Table 1.

(5) Performance of experiment.

Take 2 mg from each colonies of 24 hours standard agar slant culture of Staph. c. pyogen. aureus, Prot. vulgaris, Hauser, Bac. typhosus and Vib.

Table 1.

number of C atom	acids	chemical formulae	pH of $N/10$	pH of $N/100$	pH of $N/1000$	smell	taste
C_1	Formic	$H\cdot CO_2H$	2.32	2.83	3.33	on $N/100$ slightly sour	on $N/100$ slightly sour
C_2	Acetic	$CH_3\cdot CO_2H$	2 87	3.37	3.87	〃	〃
C_3	Propionic	$CH_3\cdot CH_2\cdot CO_2H$	2.93	3.43	3.93	〃	〃
C_4	Butyric	$CH_3\cdot (CH_2)\cdot CO_2H$	〃	〃	〃	on $N/100$ slightly unpleasant sour	〃
〃	Isobutyric	$(CH_3)_2CH\cdot CO_2H$	〃	〃	〃	〃	〃
C_5	Valeric	$CH_3\cdot (CH_2)_3\cdot CO_2H$	2.89	3.39	3.89	on $N/100$ fruitlike	〃
〃	Isovaleric	$(CH_3)_2CH\cdot CH_2\cdot CO_2H$	〃	〃	〃	〃	〃
C_6	Caproic	$CH_3\cdot (CH_2)_4\cdot CO_2H$		3.41	3 91	on $N/100$ slightly unpleasant sour	〃
〃	Isocaproic	$(CH_3)_2\cdot CH\cdot (CH_2)\cdot_2\cdot CO_2H$		〃	〃	〃	〃
C_7	Oenanthylic	$CH_3\cdot (CH_2)_5\cdot CO_2H$		3.44	3.94	〃	〃
C_8	Caprylic	$CH_3\cdot (CH_2)_6\cdot CO_2H$			4.8	on $N/1000$ slightly unpleasant sour	on $N/1000$ no taste
C_9	Pelargonic	$CH_3\cdot (CH_2)_7\cdot CO_2H$			〃	〃	〃
C_{10}	Capric	$CH_3\cdot (CH_2)_8\cdot CO_2H$			〃	〃	〃

cholerae, and put each 2 mg into 10 c.c. of sterilised physiological NaCl solution and make 4 microorganic suspensions.

Make these 4 species of suspensions at 20°C in incubator (± 0.5°C), also keep each series of 10 c.c. taken from reagents at 20°C.

Put 0.1 c.c. of suspension into 10 c.c. of reagent and mixed them homogeneously.

At the certain time I inoculate microorganism from reagents into standard bouillon with the certain platinum loop and cultivate them for 48～72 hours in incubator.

Alive or death of microorganisms are determined by the turbidity of stadard bouillon culture.

Results are as following tables.

Table 2, Sterilising action in *N*/10

Acids	pH	Surviving period																				
		Staph c pyogen. aur						Prot. vulgar. H.						Bac. typhos.						Vib. choler.		
		Minut					Hour	Minut						Minut						Minut		
		20	30	45	60	90	2	5	10	15	20	30	45	10	15	20	30	45	60	2	2.5	5
Formic	2.3	+	±	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	-	−	−	−
Acetic	2.9	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	−	−	±	−	−
Propionic	〃	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	−	−	±	−	−
Butyric	〃	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	−	−	±	−	−
Isobutyric	〃	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	+	−	−
Valeric	〃	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	±	−	−	−	−	−
Isovaleric	〃	+	+	+	+	+	−	+	+	+	+	+	−	++	+	+	+	±	−	±	−	−
Control	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\+ ···· alive − ···· perished ± ···· sometimes alive and sometimes perished

Table 3. Sterilising action in *N*/100 of Fatty acids.

Acid				Formic acid	Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	Valeric acid	Isovaleric acid	Caproic acid	Isocaproic acid	Oenanthylic acid	Control
pH				2.8	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.44	
Surviving period	Staph. c. pyogen. aur.	Minut	1	+	+	+	+	+	+	+	+	+	+	+
			2.5	+	+	+	+	+	+	+	+	+	±	+
			5	+	+	+	+	+	+	+	+	+	−	+
		Hour	2	+	+	+	+	+	+	+	+	+	−	+
			3	−	+	+	+	+	+	+	±	+	−	+
			6	−	+	+	+	+	+	+	−	+	−	+
			9	−	+	+	+	+	+	+	−	−	−	+
			12	−	−	−	−	+	−	±	−	−	−	+
			24	−	−	−	−	−	−	−	−	−	−	+

Surviving period	Prot. vulgar. H.	Minut	1	+	+	+	+	+	+	+	+	+	−	+
			2.5	+	+	+	+	+	+	+	+	+	−	+
			5	+	+	+	+	+	+	+	+	+	−	+
			60	+	+	+	+	+	+	+	+	+	−	+
			90	−	+	+	+	+	+	+	−	+	−	+
		Hour	2	−	+	+	+	+	±	+	−	+	−	+
			3	−	−	−	−	+	−	±	−	−	−	+
			6	−	−	−	−	−	−	−	−	−	−	+
			9	−	=	−	−	−	−	−	−	−	−	+
	Bac. typhosus	Minut	1	+	+	+	+	+	+	+	+	+	±	+
			2.5	+	+	+	+	+	+	+	+	+	−	+
			5	+	+	+	+	+	+	+	+	+	−	+
			60	+	+	+	+	+	+	+	+	+	−	+
			90	+	+	+	+	+	+	+	+	+	−	+
		Hour	2	−	+	+	+	+	+	+	−	+	−	+
			3	−	±	±	±	+	−	+	−	−	−	+
			6	−	−	−	−	±	−	−	−	−	−	+
			9	−	−	−	−	−	−	−	−	−	−	+
	Vib. cholerae	Minut	1	+	+	+	+	+	+	+	+	+	−	+
			2.5	−	+	+	+	+	+	+	±	+	−	+
			5	−	+	+	+	+	+	+	−	−	−	+
			10	−	+	+	+	+	+	+	−	−	−	+
			15	−	+	+	+	+	±	+	−	−	−	+
			20	−	−	−	−	+	−	±	−	−	−	+
			30	−	−	−	−	−	−	−	−	−	−	+

Table 4. Sterilising action in $N/1000$ of Monobasic Fatty acids.

Acids				Formic acid	Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	Valeric acid	Isovaleric acid	Caproic acid	Isocaproic acid	Oenanthylic acid	Caprylic acid	Pelargonic acid	Caprinic acid	Control
pH				3.3	3.9	3.9	″	″	″	″	3.9	″	3 94	4.8	″	″	
Surviving period	Staph. c. pyogen. aur.	Minut	1.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+
			20	+	+	+	+	+	+	+	+	+	+	±	±	±	+
			30	+	+	+	+	+	+	+	+	+	+	−	−	−	+
		Hour	9	+	+	+	+	+	+	+	+	+	+	−	−	−	+
			12	+	+	+	+	+	+	+	+	+	±	−	−	−	+
			24	−	+	+	+	+	+	+	±	+	−	−	−	−	+
			36	−	±	±	±	+	−	+	−	+	−	−	−	−	+
			48	−	−	−	−	+	−	±	−	−	−	−	−	−	+
			72	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	Prot. vulgaris, H.	Minut	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
			2.5	+	+	+	+	+	+	+	+	+	+	+	±	±	+
			5	+	+	+	+	+	+	+	+	+	+	−	−	−	+
			10	+	+	+	+	+	+	+	+	+	+	−	−	−	+
		Hour	6	+	+	+	+	+	+	+	+	+	+	−	−	−	+
			9	−	+	+	+	+	+	+	±	+	−	−	−	−	+
			12	−	+	+	+	+	+	+	−	±	−	−	−	−	+
			24	−	−	−	−	±	−	±	−	−	−	−	−	−	+
			36	−	−	−	−	−	−	−	−	−	−	−	−	−	+

Surviving period																
Bac. typhosus	Minut	2.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		5	+	+	+	+	+	+	+	+	+	+	±	±	−	+
		10	+	+	+	+	+	+	+	+	+	+	−	−	−	+
	Hour	6	+	+	+	+	+	+	+	+	+	+	−	−	−	+
		9	±	+	+	+	+	+	+	±	+	±	−	−	−	+
		12	−	+	+	+	+	+	+	−	+	−	−	−	−	+
		24	−	+	+	+	+	±	+	−	−	−	−	−	−	+
		36	−	−	−	−	+	−	±	−	−	−	−	−	−	+
		48	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Vib. cholerae	Minut	1	+	+	+	+	+	+	+	+	+	+	−	−	−	+
		2.5	+	+	+	+	+	+	+	+	+	+	−	−	−	+
		5	+	+	+	+	+	+	+	+	+	−	−	−	−	+
		15	+	+	+	+	+	+	+	+	+	−	−	−	−	+
		20	+	+	+	+	+	+	+	−	+	−	−	−	−	+
		30	±	+	+	+	+	+	+	−	±	−	−	−	−	+
		45	−	+	+	+	+	+	+	−	−	−	−	−	−	+
		60	−	+	+	+	+	−	+	−	−	−	−	−	−	+
		90	−	−	−	−	+	−	−	−	−	−	−	−	−	+
	Hour	2	−	−	−	−	−	−	−	−	−	−	−	−	−	+

We know following facts by results noted in Table 2. 3. 4.

The sterilising power of formic acid (C_1) is stronger than any of acids from C_2 to C_5 on each concentration.

From caproic acid (C_6) according to the increase of the number of C atom, the solubility of acids to water greatly deminishes, but the sterilising power increases very strongly. Caprylic acid (C_8), pelargonic acid (C_9) and capric acid (C_{10}) are very strong, above all capric acid has the strongest sterilising power.

(6) Colleration of sterilising action between Normal and Iso compound

Table 5. Colleration of sterilising action between Normal and Iso-Fatty acids affecting on microorganisms.

Butyric } Isobutyric   Valeric } Isovaleric   Caproic } Isocaproic   These 3 series of acids have th samepH on $N/10$. $N/100$. $N/1000$. According to this, the difference of sterilising power is not due to pH

Suspension 0.1 c.c. added	Staph. c. pyogenes aureus													Proteus vulgaris, Hauser.												
	$N/10$				$N/100$					$N/1000$				$N/10$				$N/100$					$N/1000$			
Surviving period	Minut			Hour	Hour					Hour				Minut				Minut		Hour			Hour			
	45	60	90	2	3	6	9	12	24	24	36	48	72	15	20	30	45	60	90	2	3	6	9	12	24	36
Butyric acid	+	+	−	−	+	+	+	−	−	+	±	−	−	+	+	−	−	+	+	+	−	−	+	+	−	−
Isobutyric "	+	+	+	−	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+	−	+	+	+	−
Valeric "	+	+	−	−	+	+	+	−	−	+	−	−	−	+	±	−	−	+	+	±	−	−	+	+	−	−
Isovaleric "	+	+	+	−	+	+	+	±	−	+	+	±	−	+	+	+	−	+	+	+	±	−	+	+	±	−
Caproic "					±	−	−	−	−	±	−	−	−					+	−	−	−	−	+	−	−	−
Isocaproic "					+	+	−	−	−	+	±	−	−					+	+	±	−	−	+	±	−	−
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+

Suspension 0.1 c.c. added	Bac. typhosus														Vib. cholerae											
	$N/10$				$N/100$					$N/1000$					$N/10$		$N/100$					$N/1000$				
Surviving period	Minut				Minut	Hour				Hour					Minut		Minut					Minut				Hour
	20	30	45	60	90	2	3	6	9	9	12	24	36	48	1	2.5	2.5	5	15	20	30	30	45	60	90	2
Butyric acid	+	+	−	−	+	+	±	−	−	+	+	+	−	−	±	−	+	+	+	−	−	+	+	+	−	−
Isobutyric "	+	+	+	−	+	+	+	±	−	+	+	+	+	−	+	−	+	+	+	+	−	+	+	+	+	−
Valcric "	+	±	−	−	+	+	−	−	−	+	+	±	−	−	−	−	+	+	±	−	−	+	+	±	−	−
Isovaleric "	+	+	±	−	+	+	+	−	−	+	+	+	±	−	±	−	+	+	+	±	−	+	+	+	−	−
Caproic "					+	−	−	−	−	±	−	−	−	−			+	−	−	−	−	−	−	−	−	−
Isocaproic "					+	+	−	−	−	+	+	−	−	−			+	+	−	−	−	+	−	−	−	−
Contral	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Suspension 0.5 c.c. added	Bac. typhosus													Vib. cholerae												
	$N/10$			$N/100$					$N/1000$					$N/10$			$N/100$					$N/1000$				
Surviving period	Minut			Minut	Hour				Hour					Minut			Minut					Minut			Hour	
	45	60	90	90	2	3	6	9	12	24	36	48	72	1	2.5	5	10	15	20	30	45	25	60	90	2	3
Butyric acid	+	−	−	+	+	+	−	−	+	+	±	−	−	+	−	−	+	+	+	−	−	+	+	+	−	−
Isobutyric "	+	±	−	+	+	+	+	−	+	+	+	+	−	+	±	−	+	+	+	+	−	+	+	+	+	−
Valeric "	+	−	−	+	+	−	−	−	+	+	−	-	−	+	−	−	+	+	+	−	−	+	+	−	−	−
Isovaleric "	+	±	−	+	+	+	±	−	+	+	+	−	−	+	±	−	+	+	+	+	−	+	+	+	−	−
Caproic "				+	±	−	−	−	±	−	−	−	−				±	−	−	−	−	+	−	−	−	−
Isocaproic "				+	+	+	±	−	+	+	−	−	−				+	+	−	−	−	+	+	−	−	−
Control	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+

Suspension 0.5 c.c. added	Staph. c. pyogenes aurcus														Proteus vulgaris, Hauser											
	$N/10$			$N/100$						$N/1000$					$N/10$			$N/100$					$N/1000$			
Surviving period	Minut	Hour		Hour						Hour					Minut			Minut	Hour				Hour			
	90	2	3	3	6	9	12	24	36	24	36	48	76	96	30	45	60	90	2	3	6	9	12	24	36	48
Butyric acid	+	−	−	+	+	+	+	−	−	+	+	+	−	−	+	−	−	+	+	±	−	−	+	+	−	−
Isobutyric "	+	+	−	+	+	+	+	±	−	+	+	+	±	−	+	+	−	+	+	+	+	−	+	+	+	−
Valeric "	±	−	−	+	+	+	+	−	−	+	+	−	−	−	+	−	−	+	+	±	−	−	+	+	−	−
Isovaleric "	+	+	−	+	+	+	+	±	−	+	+	+	−	−	+	±	−	+	+	+	±	−	+	+	+	−
Caproic "				+	−	−	−	−	−	+	−	−	−	−				+	−	−	−	−	+	−	−	−
Isocaproic "				+	+	−	−	−	−	+	+	−	−	−				+	+	−	−	−	+	±	−	−
Control	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(7) Sterilising action at the same pH of mono basic fatty acids.

To know the sterilising action of monobasic fatty acids and strong mineral acids on the same pH, I made solutions of pH 3.0 and pH 4.0 and examined.

The results are noted on Table 6.

Table 6. Sterilising action at the same pH of monobasic fatty acids.

Acids pH 3.0	Surviving period																								
	Staph. c. pyogen. aur.					Prot. vulgar. H.						Bac. typhosus					Vid. cholerae				
	Hour					Minut			Hour			Minut	Hour				Minut				
	2	3	6	12	24	45	60	90	2	3	6	90	2	3	6	9	1	2.5	5	15	20
Formic acid	+	+	−	−	−	+	+	+	−	−	−	+	+	±	−	−	+	+	+	−	−
Acetic 〃	+	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−
Butyric 〃	+	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−
Isobutyric 〃	+	+	−	−	−	+	+	−	−	−	−	+	+	−	−	−	+	+	−	−	−
Valeric 〃	+	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−
Isovaleric 〃	+	+	−	−	−	+	+	−	−	−	−	+	+	−	−	−	+	+	−	−	−
HNO_3	+	+	+	±	−	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−
HCl	+	+	+	±	−	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Acids	pH	Surviving period																	
		Staph. e. pyogen. aur.									Prot. vulgar. H.								
		Minut		Hour							Minut			Hour					
		20	30	12	24	36	48	72	96	120	1	2 5	5	6	9	12	24	36	48
Formic acid	4.0	+	+	+	+	+	+	±	−	−	+	+	+	+	+	+	+	−	−
Acetic 〃	〃	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−
Propionic 〃	〃	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−
Butyric 〃	〃	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−
Isobutyric 〃	〃	+	+	+	+	+	±	−	−	−	+	+	+	+	+	+	±	−	−
Valeric 〃	〃	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−
Isovaleric 〃	〃	+	+	+	+	+	±	−	−	−	+	+	+	+	+	+	±	−	−
Caproic 〃	〃	+	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	−
Isocaproic 〃	〃	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−
Oenanthylic 〃	〃	+	−	+	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−
Caprylic 〃	4.8	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
Pelargonic 〃	〃	±	−	−	−	−	−	−	−	−	+	±	−	−	−	−	−	−	−
Capric 〃	〃	±	−	−	−	−	−	−	−	−	+	±	−	−	−	−	−	−	−
HNO_3	4.0	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
HCl	〃	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Acids	pH	Surviving period																			
		Bac. typhosus										Vib. cholesae									
		Minut			Hour							Minut							Hour		
		5	10	15	9	12	24	36	48	72	96	1	2.5	5	20	30	60	90	2	3	6
Formic acid	4.0	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	−
Acetic 〃	〃	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−	−

Propionic	acid	4 0	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−	−
Butyric	〃	〃	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−	−
Isobutyric	〃	〃	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	−	−	−
Valeric	〃	〃	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−	−
Isovaleric	〃	〃	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	−	−	−
Caproic	〃	〃	+	+	+	+		−	−	−	−	−	+	+	+	+	−	−	−	−	−	−
Isocaproic	〃	〃	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	±	−	−	−	−
Oenanthylic	〃	〃	+	+	+	+	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−
Caprylic	〃	4 8	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pelargonic	〃	〃	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−
Capric	〃	〃	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HNO_3		4 0	+	+	+	+	+	+	+	+	±	−	+	+	+	+	+	+	+	+	±	−
HCl		〃	+	+	+	+	+	+	+	+	±	−	+	+	+	+	+	+	+	+	±	−
Control			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

By above experiments we know next facts. On the same pH of acids, the sterilising action of formic acid (C_1) is the weakest and acid from C_2 to C_6 have nearly the same sterilising power. These 6 acids have more stronger sterilising power than that of formic acid. From C_7 the sterilising power of acids increases suddenly very strong. Especially acids of C_8, C_9, C_{10}, are extraordinally strong. Accordingly undissociated molecules have great collera-tion on the sterilising action of saturated monobasic fatty acids. And this fact is distinct according to the increasement of C atom.

On the same pH, the sterilising action of monobasic fatty acids have the more stronger sterilising power than strong mineral acids.

(8) Sterilising action of anion of saturated monobasic fatty acids.

Acids are all dilute watery solution under *N*/10. Accordingly the sterilising action of acids can be thought the action of pH, undissociated molecule and anion. To know the sterilising action of anions (from formic to isocaproic), I made *N*/100 of the Na salt having the same anion of acids. (*N*/1000, ...Oenanthylic, Caprylic, Pelargonic, Capric) Results are as following table.

Table 7.

Surviving period / Na-salts		Staph. c. pyogen. aur.					Prot., vulgar. H.						Bac. typhosus						Vib. cholerae					
		Day					Day						Day						Minut		Hour			
		4	5	6	7	8	2	3	4	5	6	7	2	3	4	5	6	7	60	90	2	3	6	6
Formate	*N*/100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetate	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Propionate	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Butyrate	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Isobutyrate	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Valerate	〃	+	+	+	+	±	+	+	+	+	+	±	+	+	+	+	+	±	+	+	+	+	+	±
Isovalerate	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Caproiate	N/100	+	+	+	+	±	+	+	+	+	+	±	+	+	+	+	+	−	+	+	+	+	+
Isocaproiate	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oenanthylate	N/1000	+	+	+	+	−	+	+	+	+	±	−	+	+	+	+	−	−	+	+	+	+	+
Caprylate	〃	+	+	±	−	−	+	±	−	−	−	−	+	+	±	−	−	−	+	+	+	+	−
Pelargonate	〃	+	+	±	−	−	+	±	−	−	−	−	+	+	±	−	−	−	+	+	+	+	−
Caprinate	〃	+	+	±	−	−	+	±	−	−	−	−	+	+	±	−	−	−	+	+	+	±	−
Control		+	+	+	+	−	+	+	+	−	−	−	+	+	+	+	−	−	+	+	+	+	+

By above experiment we know next facts.

Anions of each acids from C_1 to C_5 have no sterilising power, but anions of acids—C_8, C_9, C_{10}—, have very weak sterilising power respectively.

(9) Relation to sterilising action of molecular concentration and pH.

Results are as following table.

Table 8. Colleration of sterilising action between molecular concentration and pH.

Acids	Normal	Surviving period / pH	Staph. c. pyoger aur							Prot. vulgar.						Vib. choler.		
			Minut				Hour			Minut						Minut		
			30	45	60	90	2	3	6	15	20	30	45	60	90	1	2.5	5
Formic acid	1/100	2.8	+	+	+	+	+	−	−	+	+	+	+	+	−	+	−	−
Acetic 〃	1/10	2.9	+	+	+	−	−	−	−	+	+	−	−	−	−	±	−	−
Propionic 〃	〃	〃	+	+	+	−	−	−	−	+	+	−	−	−	−	±	−	−
Butyric 〃	〃	〃	+	+	+	−	−	−	−	+	+	−	−	−	−	±	−	−
Valeric 〃	〃	〃	+	+	+	−	−	−	−	+	+	−	−	−	−	±	−	−

Acids	Nolmal	Surviving period / pH	Staph. c. pyogen. aur.									Prot. vulgar. H							Vib. cholerae					
			Minut				Hour					Minut			Hour				Minut					
			2.5	5	20	30	3	6	9	12	24	2.5	5	60	2	3	6	9	2 5	5	15	20	30	45
Formic acid	1/1000	3.3	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	±	−
Acetic 〃	1/100	3.4	+	+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	+	+	+	−	−	−
Propionic 〃	〃	〃	+	+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	+	+	+	−	−	−
Butyric 〃	〃	〃	+	+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	+	+	+	−	−	−
Valeric 〃	〃	〃	+	+	+	+	+	+	±	−	−	+	+	+	±	−	−	−	+	+	±	−	−	−
Caproic 〃	〃	〃	+	+	+	+	±	−	−	−	−	+	+	+	−	−	−	−	+	−	−	−	−	−
Oenanthylic 〃	〃	〃	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Capric 〃	N/1000	4.8	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Control			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Formic acid N/100······pH 2.8, N/1000······pH 3.3

Acetic, Propionic, Butyric, Valeric, Caproic, } acid N/10 ·· ···pH 2.9, N/100 ······pH 3.4

If the sterilising action was determined only by pH, then the sterilising

power of $N/100$ or $N/1000$ of formic acid must be stronger than those of $N/10$ or $N/100$ of acetic, propionic, butyric, valeric, carpoic acid respectively. But facts are quite contrary. And these anions have no sterilising power.

By these facts we hnow that the molecular concentration of fatty acids have competent sterilising power on microorganism.

Comparing the sterilising action of the same pH and the same molecular concentration of saturated monobasic fatty acids, caproic acid and oenanthylic acid are stronger than any of acids from formic to isovaleric acid.

Above all caprylic (C_8), pelargonic (C_9), capric (C_{10}) acids (…$N/1000$, pH 4.8) are extraordinary stronger than any of acids from formic to caproic acids (…$N/100$, pH 3.4).

These anions have very weak sterilising power. By above facts slerilising action of caprylic acid, pelargonic acid and capric acid are due to the undissociated molecule.

Conclusion

I studied the sterilising action of 13 kinds of saturated monobasic fatty acids from formic (C_1) to capric (C_{10}), on putrifactive bacteria, Bac. typhosus and Vib. cholerae.

Molecular concentration of these acids are more dilute watery solution than $N/10$ respectively.

Results are as follows:

(1) In the same molecular concentration, the sterilising power of formic acid (C_1) is stronger than any other acids (C_2) to isovaleric (C_5). 6 acids—from acetic to isovaleric—, have neary the same sterilising power.

(2) According to the increase in the number of C atom, from caproic acid the solubility of reagents to water greatly diminishes, but the sterilising power becomes greatly increase. Caprylic (C_8), pelargonic (C_9), capric (C_{10}), these 3 acids have specially very strong sterilising power.

(3) The sterilising action of lower fatty acid such as formic to isovaleric (C_5) is due to pH chiefly, but the concentration of undissociated molecules also gives somewhat strong effect upon sterilising action.

(4) Anions of saturated monobasic fatty acids have no sterilising power or have very weak sterilising power.

(5) The sterilising action of 3 acids, caprylic, pelargonic, capric, is chiefly due to the action of undissociated molecule. pH and anions of these acids have subsiduary action.

(6) Normal compounds have stronger sterilising power than Iso compounds.

(7) Mineral acids such as HNO_3, HCl, H_2SO_4 and H_3PO_4 (ortho) have stronger sterilising power than any of saturated monobasic fatty acids from formic to isocaproic acid on the same molecular concentration.

(8) Saturated monobasic fatty acid have stroger sterilising power than any of mineral acid such as HNO_3, HCl, H_2SO_4, H_3PO_4, on the same pH.

I express profound thanks for Dr. Y. Tohyama and Dr. S. Kojima for their kind advices for the experiment.

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Über die Protease und Amylase des Blutes der Seidenraupe (*Bombyx Mori*, L.).

Von

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Um zu einer Aufklarung über die Ernahrung, die Physiologie und die Phathologie der Seidenraupe zu kommen, sind selbstverstandlich Forschungen über das Blut der Larve unerlasslich. Bezüglich der chemischen Bestandteile des Blutes sind bereits von verschiedenen Seiten studien angestellt worden, aber es ist noch wenig über die darin vorkommenden Enzyme bekannt. Einige Forscher haben im Blute der Larve der Seidenraupen Tyrosinase, Invertase, Katalase, Protease und Amylase qualitativ nachgewiesen. Verf. möchte nun über seine Forschungen über die beiden letztgenannten Enzyme berichten.

I. Über die Protease.

(1) Feststellung der Proteasewirkung :— Man erhalt das Blut der Larven indem man ihnen die Füsse abschneidet. Das austrende Blut wird in einem Gefäss mit Toluol gesammelt und sofort gebraucht. Als Pufferlösungen dienten M/10 Zitrat-, M/15 Phosphat- und M/10 Glykokoll-Gemische nach

Sörensen. Als Substrat wurde 0.5%ige Kaseinlösung von der pH 10.92, die durch Lösen von 5 g Kasein in 10 ccm $N/10$ Natronlauge und einer kleinen Menge Wasser und weiter mit Wasser auf 1 Litre aufgefüllt wurde, benutzt. Die Substratlösung wurde mit der Pufferlösung und dem mit 0.85%iger NaCl-Lösung verdünntem Blut gemischt. Nach einer bestimmten Zeit wurde das ungespaltene Kasein mit 1.5%iger Trichloressigsäure gefällt und nephelometrisch bestimmt.

(2) Einfluss der Aciditat auf die Enzymwirkung :- In folgendem gibt der Verf. ein Beispiel aus den zahlreichen Ergebnissen, wie er sie im Journ. Agr. Chem. Soc. Japan (Japanisch) dargestellt hat.

1 ccm 0.5%ige Kaseinlösung + 3 ccm Pufferlösung + 1 ccm mit 0.85%iger NaCl-Lösung. fünffach verdunntes Blut. Temp. 22°, Wirkungsdauer 19 Stunden.

pH	mg gespaltenes Kasein	Kaseinspaltung %	pH	mg gespaltenes Kasein	Kaseinspaltung %
1.54	1.61	32.2	6.66	0.99	19.8
1.91	1.67	33.4	7.17	1.08	21.6
2.36	2.17	43.4	7.71	1.14	22.8
3.11	1.58	31.6	8.52	1.51	30.2
3.82	0.44	8.8	8.93	1.50	30 0
6.24	0.894	17.8	9.54	0.84	16.8

(3) Einfluss der Temperatur auf die Enzymwirkung :-

Ein Beispiel: 1 ccm 0.5%ige Kaseinlösung + 2 ccm Pufferlösung + 1 ccm 4fach verdünntes Blut.

pH	2.38		8.52	
Versuchszeit	65 Min.		55 Min.	
Temp. (°C)	mg gespaltenes Kasein	Kaseinspaltung %	mg gespaltenes Kasein	Kaseinspaltung %
8	0.12	3.0	—	—
20	0.61	15.3	0.24	6.0
25	0.69	17.3	0.45	11.3
30	0.79	19.8	0.54	13.5
35	0.95	23.9	0.63	14.8
40	1.12	28.0	0.77	19.3
45	0.84	21.0	0.65	14.0
50	0.63	15.8	0.51	12.3
65	0 52	13.0	0.12	3.0
15	0.20	5.0	0.05	1.3

(4) Beziehungen zwischen den Rassen und dem Wachstum der Seidenraupen und der Wirksamkeit der Protease :-

2 ccm 0.5%ige Kaseinlösung + 3 ccm Zitrat-salzsäuregemisch + 1 ccm 5fach verdünntes Blut. Temp. 31°. pH 2.38. Versuchszeit 24 Stunden.

In den 5ten Alter	1ter Tag		3ter Tag		6ter Tag	
	gespaltenes Kasein		gespaltenes Kasein		gespaltenes Kasein	
Rasse	mg	%	mg	%	mg	%
Japan-110	4.75	51 9	—	—	4.75	51.9
Japan-Gin. × Europa-7	4.53	49.6	4.14	45 3	4.14	45.3
Japan-110 × Europa-7	4.63	50.7	4.22	4.22	4.65	50.3
China-7 × Japan-Gin.	4.34	47,5	4.34	47.5	4.70	51.4
Europa-7 × Japan-110	4.61	50 4	—	—	4.85	53.1

(5) Der Unterschied zwischen der Kaseinspaltbarkeit des Bluts bei Mannchen und Weibchen der Larve :–

2 ccm 0.5%ige Kaseinlösung + 3 ccm Pufferlösung + 1 ccm 5fach verdunntes Blut. pH 2.38, Versuchsdauer 20 Stunden.

	♀Nr. 1	♀Nr. 2	♀Nr. 3	♀Mittel	♂Nr. 1	♂Nr. 2	♂Nr. 3	♂Mittel
mg gespaltenes Kasein	4.33	4 25	4 29	4 29	4.76	4 72	4.81	4 76
Kaseinspaltung %	43.3	42.5	42.9	42 9	47 6	47.2	48 1	47.6

II. Über die Amylase.

(1) Feststellung der Amylasewirkung :– Die Gewinnung des Bluts und der Gebrauch der Pufferlosung waren dieselben wie in dem Experiment über die Protease. Zur Mischung der 5%igen Starkelosung und der Pufferlosung wurde das mit 0.85%iger NaCl-Losung verdünnte Blut addiert und nach bestimmter Versuchsdauer der entsandene, reduzierende Zucker mittels der Methode von Bertrand bestimmt.

(2) Einfluss der Aciditat auf die Amylasewirkung :–

Ein Beispiel: 3 ccm 5%ige stärkelosung + 5 ccm Pufferlösung + 1 ccm 3fach verdünntes Blut. Temp. 31°, Wirkungeszeit 24 Stunden.

pH	ccm 0.5% $KMnO_4$	mg Maltose	Spaltung %	pH	ccm 0.5% $KMnO_4$	mg Maltose	Spaltung %
5.20	1.6	14.3	6 5	7.17	5.0	45.4	20.7
5.81	4.2	38.1	17 4	7.60	3.2	28.7	13.1
6.18	6.0	55.3	25.2	8.04	2.4	21.6	9.8
6.61	6.2	56 5	25.8	8.52	2.2	19.8	9.0

(3) Einfluss der Temperatur auf die Enzymwirkung :–

Ein Beispiel: 4 ccm 5%ige Stärkelösung + 4 ccm Pufferlösung + 2 ccm 5fach verdünntes Blut. pH 6.18, Versuchsdauer 90 Min.

Temp. °C	20	25	30	35	40	45	55	65	75
ccm 0.1% $KMnO_4$	3.0	3.6	4.4	4.8	4.0	1.6	0.9	0.4	0.2

(4) Beziehungen zwischen den Rassen und dem Wachstum der Seidenraupen und der Wirksamkeit der Amylase :–

Angewandt: 3 ccm 5%ige Stärkelösung, 4 ccm Phosphatgemisch, 1 ccm 5fach verdünntes Blut. pH 6.18, Temp. 31°, Versuchszeit 24 Stunden.

In den 5ten Alter	Rasse	Japan-110	Japan-Gin. × Europa-7	Japan-110 × Europa-7	China-7 × Japan-Gin.	Europa-7 × Japan-110
1ter Tag	ccm 0.5% $KMnO_4$	2.3	1.5	2.0	1.6	2.0
	mg Maltose	20.7	13.0	18.0	14.3	18.0
	Spaltung %	15.7	10 2	13.7	10.9	13.7
3ter Tag	ccm 0.5% $KMnO_4$	——	2.4	3.3	8.8	——
	mg Maltose	——	21.6	29.7	77.0	——
	Spaltung %	——	16.4	22.6	58.5	——
6ter Tag	ccm 0.5% $KMnO_4$	3.2	3.8	2.9	2.9	3.3
	mg Maltose	28.8	34.2	26.1	26.1	29.1
	Spaltung %	21.9	26.0	19.1	19.1	22.6

(5) Der Geschlechtsunterschied in der Verzuckerungskraft des Bluts bei der Seidenraupe :–

Angewandt: 6 ccm 5%ige Stärkelösung, 8 ccm Pufferlösung, 1 ccm 5fach verdünntes Blut. pH 6.18, Temp. 28°, Versuchsdauer 20 Stunden.

	♀Nr. 1	♀Nr. 2	♀Nr. 3	♀Mittel	♂Nr. 1	♂Nr. 2	♂Nr. 3	♂Mittel
ccm 0.5% $KMnO_4$	2.10	2.25	2.10	2.15	1.50	1.95	2.10	1.82
mg Maltose	18.9	20.3	18.9	19.4	13.4	17.6	18.9	16.6
Spaltung %	7.2	7.7	7.2	7.4	5.1	6.8	7.2	6.4

Zusammenfassung.

(1) Die protease des Bluts der Seidenraupe zeigt zwei ausgesprochene pH-Optima, namlich pH 2.3 und pH 8.8. Das Temperaturoptimum liegt bei 40°.

(2) In der proteolytischen Wirkung des Bluts besteht kein Unterschied in den Rassen und in den Wachstumperioden der Seidenraupen, aber diese Wirkung ist bei den Mannchen etwas starker als bei den Weibchen.

(3) Die Blutamylase der Seidenraupe zeigt die optimal Aktivitat bei pH 6.5 und das Temperaturoptimum liegt bei 35°.

(4) Unter funf Rassen der Seidenraupen besteht kein Unterschied in den Wirksamkeit der Blutamylase. Die Amylasewirkung wird mit dem Wachstum der Seidenraupe starker und ist auch bei den Weibchen etwas starker als bei den Mannchen.

Über die Begünstigung des Azotobacter-Wachstums durch mineralische Stoffe aus Bodenextrakten.

Von

K. Konishi und T. Tsuge.

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(Folgende Uutersuchungen wurden im Laboratorium für Bakteriologie der Biologischen Reichsanstalt für Land- und Forstwirtschaft in Berlin-Dahlem und im Agrikulturchemischen Institut der Kaiserlichen Universität zu Kyoto, Japan, ausgeführt. Wir sprechen Herrn Oberregierungsrat Dr. C. Stapp und Herrn Dr. H. Bortels für die Anregung zu diesen Untersuchungen und fur viele Ratschläge unseren herzlichen Dank aus. Ebenso sind wir den Herren Prof. Osugi, Prof. Kimura, Dr. Iwamura, und Dr. Miyanisi für mancherlei Rat zu Dank verpflichtet.)

Die für Entwicklung und Wachstum des Azotobacters in synthetischen Nährlösungen notwendigen oder nützlichen Stoffe wurden schon oft von verschiedenen verfassern eingehend untersucht. Einige von solchen Stoffen wurden für Azotobacter als lebensnotwendig, andere als stimulierend wirkend angesehen, und Nahrlösungen, die 10 und mehr anorganische Substanzen enthielten, wurden

als geeignete Nahrboden empfohlen. So wurden von Ashby eine sehr gute synthetisch hergestellte und von Meyerhof und Burk und von Wolff ahnliche, etwas modifizierte Nahrlosungen vorgeschlagen. Jedoch vermochten nach Krzemieniewski (1901, 1907) und Christensen (1915) Reinkulturen von Azotobacter in N- freier Nahrlosung bei Gegenwart von frischer oder sterilisierter Erde mehr N aus der Luft zu fixieren als in Nahrlosung ohne Erde. Christensen hat gemeint, dass für die N-Bindung nicht nur die in der Nahrlosung gewohnlich vorhandenen Stoffe, sondern noch irgendwelche Bestandteile des Bodens notwendig seien. Bortels hat neuerdings eine erhebliche Wirkung von Erdextraktasche beobachtet. Er hat Erde mit Wasser extrahiert und den Extrakt einer Mannit Nahrlosung zugesetzt. Dann hat er gefunden, dass bei Verwendung solcher-Erdextrakte, die bel neutraler bis alkalischer Reaktion gewonnen wurden, ein sehr viel kraftigeres Azotobacterwachstum stattfindet als ohne Zusatz von solchen Auszügen aus Erdextraktaschen.

Es gibt viele Moglichkeiten, die Wirkung von Erdextrakten zu erklaren. Man glaubte, dass diese auf in den Extrakten vorhandene Salze des Eisens oder Aluminiums beruhe, oder dass Kolloide der Hydroxyde des Eisens oder Aluminiums oder die in geringer Menge vorhandenen organischen Substanzen hierfür verantwortlich zu machen seien. Wir nahmen mit Bortels an, dass sich im Erdextrakt auf die N–Bindung des Azotobacters günstig wirkende anorganische Bestandteile befinden müssen. Bortels hatte ermittelt, dass Salze des Molybdans (1930) und solche des Vanadins (bisher noch unveroffentlicht!), in geringer Menge der Nahrlosung zugesetzt, Azotobacter zu mindes tens ebeso energischer N–Bindung befahigen wie Erdextraktasche, und zwar Vanadin in etwas geringerem Masse als Molybdan.

Wir wollten nun versuchen, die Anwesenheit solcher Elemente in den Erdextrakten nachzuweisen und bedienten uns dabei der spektroskopischen Methode, da nach Lundegardh (1929) und Iwamura (1931) die Spektralanalyse eine zuverlassige Mikromethodik ist.

Wir haben 1 kg mit $CaCO_3$ versetzter Erde aus Dahlem mit 1 L aqua dest. bei 2 Atm. extrahiert, den Extrakt in der Quarzschale zur Trockene eingedampft und verascht. Aus dieser Asche wurde ein Auszug mit heissem Wasser hergestellt und, wenn notig, mit H_2SO_4 neutralisiert. Dann wurde daraus eine Nahrlosung mit Dextrose und mineralischen Salzen bereitet entsprechend der Stammlösung, die sich wie folgt zusammensetzte:

Aqua dest.	100 ccm	$MgSO_4 \cdot 7H_2O$	0.05 g
Dextrose	2.0 g	$FeSO_4 \cdot 7H_2O$	0.005 g
K_2HPO_4	0.1 g	$CaCO_3$	0 5 g

Als Versuchsgefasse dienten 750 ccm fassende Erlenmeyer-Kolben aus Jenaer Glas. Der auf Möhren-Agar gewachsene junge Stamm von Azotobac-

ter chroococcum wurde in die Nährlösungen eingeimpft, die dann 5 Tage im Brutschrank bei 27~28°C gehalten wurden. Am Ende der Versuchsdauer liessen die kolben mit Erdextraktasche eine deutliche Trübung erkennen, und der N-Gewinn betrug 3.8 mg auf 100 ccm, während die Kulturen ohne Erdextraktasche nur Spuren von Stickstoff enthielten. Ferner haben wir gleiche Versuche mit zwei Arten von Erdextraktaschen, die sich auf das Azotobacterwachstum günstig ("gut") oder nicht günstig ("schlecht") auswirkten, und einer solchen von Tschernosemboden durchgeführt. Nach 10 Tagen Versuchsdauer war der N-Gehalt der Kultur mit "guter" Erdextraktasche von 0.91 mg auf 6.75~12.79 mg und derjenige der Kultur mit "schlechter" Extraktasche auf 3.10~4.20 mg gestiegen. Ein Zusatz von Tschernosem-Asche vermochte die N-Bindung wenig zu erhöhen. Aus weiteren Versuchen ging hervor, dass die Kulturen in Nahrlösungen, die grössere Mengen von Extraktaschen der "schlechten" Erde oder des Tschernosem enthielten, ebenfalls gut wuchsen. Wahrscheinlich waren in diesen Aschen die Elmente, die der Entwicklung des Azotobacters in N-freier Nahrlösung dienlich sind, in viel geringerer Menge enthalten.

Ähnliche Untersuchungen wurden mit 12 Erdproben aus 6 verschiedenen Gegenden ausgeführt, die sich untereinander durch den Grad ihrer Fruchtbarkeit unterschieden. Bei Zusatz der Asche aus fruchtbarer Erde war der erhaltene N-Gewinn nicht immer bedeutend. Nur bei Verwendung des N armen Erdextraktes selbst fanden wir wieder den Parallelismus zwischen Fruchtbarkeit der Erde und N-Gewinn.

Die Spektralanalyse mit Hilfe des Lichtbogens nach Kimura oder Nitchie diente zur Bestimmung der Elemente der Erdextraktashe. Die hierzu notwendige Apparatur bestand aus den beiden Kohlen-Elektroden und einem Spektroskop E 3 von Adam Hilger. Als untere Elektrode benutzten wir eine Kohle von 9 mm Durchmesser, die mit einer Bohrung von 5 mm Durchmesser versehen wurde, welche die zu untersuchende Probe aufnehmen sollte. Wir hielten zwischen beiden Elektroden immer einen Abstand von 4 mm, benutzten den Gleichstrom von 110 Volt und arbeiteten gewöhnlich mit 5 Ampere. Vor den Spaltkopf wurde eine Hartmann'sche Spaltblende mit drei Öffnungen befestigt, wodurch wir 3 vergleichbare Spektrogramme, und zwar Eisen als Standard, Kohle mit Probe und Kohle allein gleichzeitig aufnehmen konnten. Die auf den Ilford-Panchromatic-Platten aufgenommenen Linienspektren wurden unter Hilger's Mikrometer beobachtet, um die Lage der den unbekannten Elementen zukommenden Linien zu bestimmen und ihre Wellenlänge zu berechnen. Zuweilen verwendeten wir zum Vergleich das Spektrogramm eines Elementes, das wir in der Probe vermuteten, in reiner Lösung. Wenn sich dann die typischen Linien deckten, so bewiesen wir

damit das Vorhandensein des fraglichen Elementes.

Nunmehr untersuchten wir die "gute" Erdextraktasche aus Berlin-Dahlem spektrographisch nach der oben geschilderten Methode. Ausser den Elementen, die in der Regel in grosseren Mengen vorkommen, konnten wir mit Bestimmtheit die nachfolgenden Elemente in sehr geringer Menge nachweisen:

Titan, Barium, Strontium, Lithium, Chrom, Vanadin, Zink, Nickel.

Es erhebt sich nun die Frage, ob in Anwesenheit von einigen Salzen dieser Elmente die N-Bindung energischer verlauft als in gewohnlicher Nahrlosung. Um Klarheit zu bekommen, liessen wir in den einzelnen Versuchsserien jeweils ein Salz eines der oben ganannten Elemente in verschiedenen Konzentrationen einwirken. Mit Hilfe der Erlenmeyer-Technik von Burk fanden wir, dass Vanadin die N-Bindung durch Azotobacter zu forden vermag, wenn es in Mengen von M/10,000~M/100,000 als Natriummetavanadat oder Vanadium-Chlorid gegeben wird, und dass den anderen obengenannten Elementen hierbei keinerlei Bedeutung zukommt. Die Reinkultur hatte 0.5~0.7 mg N in 100 ccm gebunden, bei Zusatz von Vanadin-Salz dagegen 4.3~6.7 mg.

In dem Spektrogramm des Wasserauszuges aus "guten" Erdextraktasche erschienen die Linien λ 3102, 3113, 3118, 3184 und 3185, die für die Anwesenheit des Vanadins entscheidend sind, wahrend in dem Spektrogramm der "schlechten" Erdextraktasche die Zahl der Linien abnahm, und λ 3183, 3184 und 3185 nur schwach ausgepragt waren. Bei Tschernosem waren auffalligerweise alle Vanadin-Linien verschwunden. Bei den anderen 12 Erdextraktaschen und ihren Auszügen war in einem Fall die Intensitat der letzten Vanadin-Linien sehr schwach und in den ubrigen Fallen fast unsichtbar und kaum vergleichbar.

Auf Grund dieser Ergebnisse konnen wir behaupten, dass die Menge des Erdextraktasche enthaltenen Vanadins einen wichtigen Faktor darstellt, von welchem die Grosse der N-Bindung durch Azotobacter abhangig ist.

Electro-Conductivity of Textile Fibres

By

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(Received 28, December, 1932)

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Summary.

The method of determining the electro-conductivity of the textile fibres and the influences of the treatments on the electro-conductivity of silk have been investigated with the following results.

(1) The electro-conductivity of textile fibres can be determined comparingly by the measurement of a constant electric quantity which is charged in the quadrant electrometer, in the length of time which is needed to discharge it through the test piece of the textile fibre.

(2) The electro-conductivity of raw silk which is stored in places of different moisture and temperature for two years (temp. 5～30°C, relative humidity 40～90%) can not be distinguished.

(3) The electro-conductivity of the rayon is generally larger than that of the natural silk. Among the rayons, the electro-conductivity of acetate silk is much less than that of the others.

(4) When raw silk is degummed off, the electro-conductivity of it decreases. The electro-conductivity of the scoured silk by means of soap or free caustic soda solution is greater than that of the silk scoured by enzyme such as pancreatin, therefore, if one wishes to get silk of high electric insulation, it is preferable to scoure it by enzyme.

(5) The electro-conductivity of the scoured silk by means of water of high temperature and pressure (over 121°C, two atmospheric press.) is greater than that of the silk scoured by soap or enzyme on behalf of its destruction of fibroin by the treatment under high temp. and pressure.

(6) When the scoured silk by means of soap solution is treated by NaOH of different concentration, the electro-conductivity of these silks differs, that is, the electro-conductivity of the silk treated by the more concentrated NaOH, is greater than that of the less concentration. The reason of this does not depend only on the destruction of the fibroin, but is much influenced by the sodium combined with it. This conclusion comes from the facts that. a) When the concentration of NaOH is very low ($N/400$) the strength and elongation of the treated silk is not decreased, while the electro-conductivity of it is increased compared with that of the untreated. b) Furthermore, when the treated silks with NaOH are immerced into a diluted sulphuric acid the electro-conductivity of the sulphuric acid is decreased according to the degree of concentration of NaOH which is used to treat the silk, that is, the more the sodium combined with the silk fibre the greater its power of decreasing the electro-conductivity of the sulphuric acid when the silk is immerced into it.

(7) When silk is weighted by tin-salt, the electro-conductivity of it is increased according to its degree of weighting. This increase of electro-conductivity is not caused by the destruction of the fibre, but by the weighted salt, because the strength and elongation of the silk (at rhe course of weighting) is increased to some extent by the weighting, that is to say, the destruction of the silk by weighting must occur in the storage. From this result the announcement "The tin-weighting of the silk will make it less liable to become dirty." seems to be reasonable.

Content of Vitamin C in Canned Satsuma Orange

(*Citrus unshiu, Marc.*)

A preliminary report.

By

R. SAITO.

(Received February 4, 1933)

1. Introduction.

Unshiu (Satsuma orange)–a principal mandarine of Japan, also palatable and nutritious, is liable to be infected with mould when it is preserved for a long period. This defect can adequately be removed through canning, which is a recently developed industry in this country.

There are now two different methods in canning: The one consists in treating the peeled orange with caustic alkali to dissolve the envelope of segments out, the other with acid instead of alkli. The latter so-called "acid process", was invented by B. Hamaguchi in the Institute of Dietary Science, Agricultural Department of Tokio Imperial University, and recently improved thoroughly (Jap. pat. No. 95669). The author estimated vitamin C content in Satsuma orange which was canned by the "acid process".

2. Process of Canning (Acid process).

From peeled orange each segment is detached and immersed in 10 per-

cent hydrochloric acid at 90°C. After 30~40 sec., when the pectinous substance forming the wall of segments is almost decomposed into the pectinic acid, it is drained and washed with water. The mass of vesicles is then put into a concentrated solution of salts in which the remaining hydrochloric acid can be diffused out by osmotic action. By few hours' washing with clean water, traces of hydrochlorie acid and salts can effectually be removed. A sugar solution of Bé 26° is then poured on and they are packed and sterilized for 10 min. at 100°C.

3. Chemical composition of *Citrus unshiu.*

The fresh orange used for the canning, is *Citrus unshiu* produced in Shizuoka District. The chemical composition of the fresh fruit is as follows: (the rind is taken off, pressed, filtered and analysed.)

	Total sugar in 100 c.c. gr.	Reducing sugar in 100 c.c. gr.	Free acid as citric %
in fresh juice of orange	7 133	2.345	1.10

The content of each can of the orange, here reffered, is as follows:

Total content	Fruit flesh	Sugar solution
327 gr.	224 gr.	98 c.c.

The chemical composition of the above is given below.

		Specific gravity	Total Sugar in 100 c.c. gr.	Reducing sugar in 100 c.c. gr.	Free acid as citric %
3 days after canning	Flesh	——	12.725	4.100	1.18
	Sugar soln.	——	19.325	4.275	0.59
47 " "	Flesh	——	17.100	7.075	0 92
	Sugar soln.	——	17.100	7.075	0.69
80 " "	Flesh	1.061 1.062	——	——	——
	Sugur soln.	1.062 1.063	——	——	——
360 " "	Flesh	——	8.750	5 750	0.76
	Sugar soln.	——	8.750	5.750	0.76

4. Animal Experiment.

As animals for this purpose, guinea-pig weighing about 250 grms. were

used. The author used a basal diet of oats and wheat bran with milk (50 c.c. per head per day) autoclaved at 120°C for one hour and supplemented with 5~6 drops of cod-liver oil.

The samples tested were prepared by filtering the pressed juice of canned orange (the mass of vesicles) which had been packed three months ago (from 100 grs. of the mass of vesicles 70~75 c.c. of pressed juice was obtained). Preliminary feeding period lasted 7~17 days, during which the above basal ration was supplemented with cabbege. Throughout the tests male were used.

(a) Preventive experiment.

The following table shows the result when 5~8 c.c. of pressed juice of the canned orange (mass of vesicles) were given per day.

No.	Pressed juice given, c.c.	Days of experiment	Body weight of animal			Symptom of Scurvy
			at the beginning of test	maximum	after the test	
2	0	23	268	320	198	severe
10	0	28	259	304	211	—
3	5	58	241	436	423	no
4	5	58	284	304	299	〃
5	8	58	282	465	454	〃
6	8	58	281	394	359	〃
7	8	58	244	443	436	〃

The data obtained in this experiment show that 5 c.c. per day is sufficient to prevent a guinea-pig from scurvy. In the course of this test no symptom of scurvy could be recognized, while the control animals fed solely on vitamin C free diet, showed clearly the symptoms of scurvy even after 12 days. Decline of body weight occurred and death followed soon after. On post-mortem examination all the sign of scurvy were fully recognized.

(b) Curative experiment.

After feeding two guinea-pigs on vitamin C free basal diet, when the symptom of scurvy was distinctly observed, 10 c.c. of the pressed juice of the canned orange (mass of vesicles) were administered. One recovered from the desease, gaining body weight rapidly and the sign of complete recovery was also anatomically affirmed. The experimental result is as follows:

No.	Days of feeding on vitamin C free-diet	Days of pressed juice given	Body weight of Animal			Symptom of Scurvy
			Before test	at Beginning of test	after test	
1	19	31	277	224	188	slightly
8	17	40	241	228	359	—

5. Summary.

(1) Canned mandarine orange (*Citrus unshiu*) contains rich amount of vitamin C equalling to more than 1/3 of fresh lemon or orange juice.

(2) According to Dr. R. Fujimaki,[1] fresh *Unshiu* contains about 3/8 of vitamin C of fresh lemon juice and Y. Iwasaki[2] 4∼5 c.c. of fresh juice of *Unshiu* is sufficient to cure a gninea-pig of scurvy.

(3) From these investigations, the author will maintain that vitamin C content in fresh *Unshiu* can hardly be affected by canning where acid process be usd. This result coincides well with Delf's[3] experiment on the vitamic C content of canned orange.

The author wishes to express his best thanks to Dr. U. Suzuki for the guidance of this work and also to Mr. K. Murai for his assistance.

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Investigation on the Influence of Aerial-Earth Circuit on the Biological Activities.

I. Influence on Azotobacter chroococcum.

By

Arao Itano, Ph. D.

(Received February 6, 1933)

The influence of aerial-earth circuit on the biological activties was investigated, and as the first report, the results obtained with Azotobacter chroococcum are presented in this paper.

The term "aerial-earth circuit" is employed here to designate the circuit between the atmosphere and the earth.

Hitherto the most biological investigations have been undertaken independent of the aerial-earth circuit or in other words, under the insulated condition. It is especially true with those concerned the microorganisms. Under the

usual laboratory conditions, the micoorganisms are insulated from the aerial-earth circuit so that the results obtained through such the procedure may not be the same as those in the closed circuit. Again it seems to be more evident in cases of the soil microorganisms whose habitat is the soil where the influence of such circuit as well as the earth potential is presumably marked.

In view of these considerations, it was attempted to ascertain experimentally the influence of the aerial-earth circuit first on Azotobacter chroococcum which is well known organism for its interesting physiological activity, namely the fixation of atmospheric nitrogen.

Experimental Procedure.

An acclamatized, young culture of Azotobacter chroococcum in Ashby's liquid medium was used to inoculate six flasks out of eight Erlenmyer flasks (250 c.c. volume) which contains 100 c.c. af Ashby's medium of the following properties, shown in Table I and II, in each, and the flasks were treated as described below:

Table I. Chemical Properties of Ashby's Liquid Medium.

Composition	Quantity
Mannitol [$C_6H_8(OH)_6$]	10.0 g.
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2
Mono-potassium phosphate (KH_2PO_4)	0.2
Sodium chloride ($NaCl$)	0.2
Calcium sulfate ($CaSO_4 \cdot 2H_2O$)	0.1
Calcium carbonate ($CaCO_3$)	5.0
Distilled water	1,000.0 c.c.

Table II. Physical Properties of Ashby's Liquid Medium.

Properties	Values
pH	7.40
Specific gravity	1.0077
Viscosity	0.98984
Specific conductance	0.00074
Surface tension	79.559 (dynes per sq. cm.)
Osmotic pressure	2.069

No previous record in regard to the physical properties of the medium is found so that they were determined realizing their significance in this investigation.

The flasks in the series were treated as follows:

Flask I. Two platinum electrodes (1.5 × 2.0 cm.) were inserted in the medium, and one of them was connected to an aerial antenna of twelve meters long and the other was earthed by a main water pipe. After the medium was inoculated with 1 c.c. of 48 hours old culture, the flask was kept in an incubator at 30°C.

Flask II. Same as Flask I except it was not inoculated with the organism, and served as a control for Flask I in regard to the nitrogen fixation.

Flask III. Same as Flask I except no earth connection was made.

Flask IV. Same as Flask I but no connection to the antenna was made.

Flask V. Same as Flask IV but only one electrode was inserted and earthed.

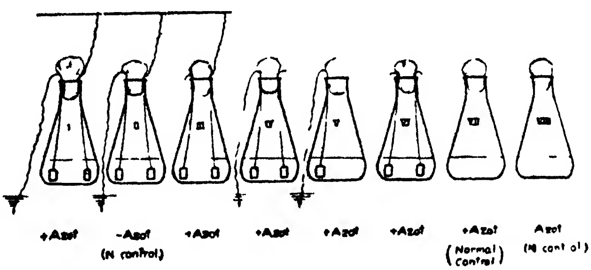
Flask VI. Same as Flask IV except no earth connection was made.

Flask VII. Same as Flask VI without insertion of any electrode.

Flask VIII. Same as Flask VII without inoculation, serving as the nitrogen control under the normal condition.

These arrangements are illustrated diagrammatically as follows:

Diagrammatical Arrangement



At the end of every 24 hours, with a few exceptions, the following observations were made:–

1. Macroscopical observations;
   a) The turbidity of culture.
   b) The formation of surface membrane.
2. Microscopical observations;
   a) The morphology of individual cell especially in regard to the cell division.
   b) The count of total cells was made by the use of hemocytometer and with a specially made cover glass in combination with Breed's ocular disc.
3. Nitrogen determination;

At every five days intervals, the nitrogen was determind by Pregel's

micro-Kjeldahl method in the course of growth, and the final determination was made by the macro-Kjeldahl method for the total nitrogen.

Results.

1. Macroscopical observations :–

The macroscopical observations were made up to the tenth day as to the turbidity and the surface membrane, and the results together with some microscopical observations are given in Table III.

Table III. Macroscopical and Microscopical Observations.

Number of Hours.	Number of Flasks. I	II	III	IV	V	VI	VII	VIII
24	T ++ M ++ Y ++ P −	T − M − Y − P −	T + M ± Y + P −	T + M + Y + P −	T + M + Y + P −	T + M ± Y + P −	T + M ± Y + P −	T − M − Y − P −
48	T ++ M ++ Y ++ P −	T, M, Y, P ibid.	T ++ M + Y + P −	T ++ M ++ Y ++ P −	T ++ M ++ Y ++ P −	T ++ M + Y + P −	T ++ M + Y + P −	T, M, Y, P ibid.
72	T +++ M +++ Y +++ P −	T, M, Y, P ibid.	T ++ M ++ Y ++ P −	T ++ M ++ Y ++ P −	T ++ M ++ Y ++ P −	T ++ M ++ Y ++ P −	T ++ M ++ Y ++ P −	T, M, Y, P ibid.
96	T ++++ M +++ Y ++++ P −	T, M, Y, P ibid.	T +++ M ++ Y ++ P −	T +++ M +++ Y +++ P −	T +++ M +++ Y +++ P −	T +++ M ++ Y ++ P −	T +++ M ++ Y ++ P −	T, M, Y, P ibid.
120	T ++++ M ++++ Y ++++ P −	T, M, Y, P ibid.	T +++ M ++ Y ++ P +	T +++ M +++ Y ++++ P +	T +++ M +++ Y ++++ P +	T +++ M ++ Y ++ P +	T +++ M ++ Y ++ P +	T, M, Y, P ibid.
(Missed one day) 168	T, M, Y ibid. P ±	T, M, Y, P ibid.	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y, P ibid.
192	T, M, Y ibid. P +	T, M, Y, P ibid.	T, M, Y ibid. P +++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y, P ibid.
216	T, M, Y ibid P ++	T, M, Y, P ibid.	T, M, Y ibid. P +++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y, P ibid.
240	T, M, Y ibid. P ++	T, M, Y, P ibid.	T, M, Y ibid. P +++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y ibid. P ++	T, M, Y, P ibid.

Notes :– T : turbidity. M : membrane. Y : young, growing cells. P : pleomorphic cells. (The cells were observed through the microscope. while the count was made.)
± : doubtful. – : no change or none. + : slight or few. ++ : marked or many. +++ : heavy or numerous. ++++ : very heavy or numerous.

Table III indicates that Flask I became turbid sooner than the rest, and those flasks which were earthed became more turbid than Flask VII which is the normal control. The formation of the surface membrane was in the same order as the turbidity. These results indicate plainly that the completion of circuit has a marked influence and also the earthing alone has a very beneficial influence on the growth of Azotobacter chroococcum. The stimulation effect was observed after 24 hours and at the end of 48 hours, the difference was noted very plainly.

2. Microscopical observations :–

The total number of cells was counted directly by means of microscope so that the morphological description of an individual cell was made at the same time. The results are given in Table IV while the conditions of cells were already noted in table III.

Table VI. Growth of Azotobacter chroococcum at Different Age.

Number of hours.	Number of Flasks.							
	I	II	III	IV	V	VI	VII	VIII
Initial.	366*	–	366*	366*	366*	366*	366*	–
24	12.112	–	5.746	6.288	5.887	5.112	5.232	–
48	35.211	–	12.154	21.408	23.183	13.883	12.957	–
72	38.028	–	18.352	24.873	28.535	18.197	17.549	–
96	44.028	–	20.985	28.676	29.661	20.197	19.605	–
120	45.915	–	26.662	30.140	30.028	27.323	26.000	–

Notes :– * Number of organisms per c.c. in thousand.

Table IV indicates that the growth of Azotobacter chroococcum was influenced markedly by closing the circuit or earthing alone. The count after five days remained practically constant and the pleomorphic cells began to appear in some of the flasks making the correct counting difficult so that no further record is given after that. Flask I exceeded all the rest and Flask IV and V were much better than Flask III, VI and VII which were very similar. The stimulation began to show its influence after 24 hours. Also as it was noted in Table III, the young, multiplying cells were most numerous in Flask I throughout the investigation and the pleomorphic cells began to appear later than the rest, which seems to indicate that the closed circuit stimulates the cell metabolism. Again Flasks IV and V were better than the others in this respect, indicating that the earthing alone had some effect,

3. **Physiological observation :–**

Although the nitrogen determinations were made at every five days intervals by Pregel's micro-Kjeldahl method, here only the results obtained at the end of fifteen days by the ordinary Kjeldahl method for the total nitrogen will be given in Table V.

Table V. Quantity of Nitrogen fixed.

Number of flasks.	Total uitrogen fixed in fifteen days per 100 c.c. of medium.
I	4 099 mg
II	——
III	2 830
IV	3 339
V	3 309
VI	2 680
VII	2 724
VIII	——

Table V indicates that much greater amount of nitrogen viz. more than one and one half times of the normal control, was fixed in Flask I where the aerial-earth circuit was closed, and even the earthing alone fixed much more than the normal control. The connection only to the antenna did not show any difference, as shown by the result in Flask III.

Discussions.

The nature of the stimulation which was observed in this investigation may be electrical in nature, but no previous investigation was undertaken in regard to this phase of problem so far as the author is aware. The previous investigations which are concerned with the influence of electricity upon the microorganism as well as other organisms, seem to confine themselves, at least experimentally to electricity applied directly to the organisms, either galvanic or static form, and the influence observed by them may be considered as electro-chemical or the secondary influence in most cases.

The amount of electric current which passes through such a system as used in this investigation is governed primarily by the conductivity of the culture medium as well as the dielectrics of the system, and it must be a very small amount judging from the physical properties of the medium. No attempt however was made at this time to measure the current since it is very difficult to determine it accurately owing to the continuous change in the culture medium as the growth of the organism progresses as well as the irregularity of the atmospheric charge and the earth potential.

Since the physical properties of calture medium seem to have the great influence upon the physiological activities of organisms, they should receive more attention in the physiological investigation than ever before as well as the physical ecological factors.

Summary.

The influence of aerial-earth circuit on Azotobacter chroococcum was investigated by cultivating it in the closed aerial-earth circuit, connecting it either to the antenna or to the earth, and comparing the results against the normal control which was grown under the ordinary laboratory condition. From the results obtained, the following summary may be made:

(1) In the closed aerial-earth circuit, Azotobacter chroococcum was influenced markedly both culturally and physiologically. Its growth was more vigorous and fixed much more nitrogen than the rest.

(2) Connection to the antenna alone did not have any influence.

(3) Connetion to the earth alone exerted better influence but was not so great as in the case of the closed circuit.

The principle underlying this investigation seems to be far reaching, scientifically as well as practically, and further investigations with various microorganisms are in progress.

Studies on The Proteins Contained in Mulberry Leaves.

Part I. On the Kinds of the Protein-Nitrogen Contained in Mulberry Leaves, and the Comparison of the Quantitities of the Protein-Nitrogen Contained in Different Parts of the Mulberry Tree.

By

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(Received February 10, 1933)

Résumé.

(1) I made the following studies concerning the proteins contained in mulberry leaves, from the view-point of a student of mulberry culture.

(2) First of all, I tried to estimate the quantities of the proteins contained in mulberry leaves by ordinary method—a method usually practised in estimating the comparative quantities of the proteins contained in vegetable seeds. The method*, as I adopted, is as follows:—

First, the leaves are extracted with distilled water,

Next, the residue left after treatment with distilled water is extracted with 10% solution of sodium chloride; and

Then, the residue left after treatments with distilled water → 10% solution of sodium chloride is extracted with 70% alcohol; and

Finally, the residue left after treatments with distilled water → 10% solution of sodium chloride → 70% alcohol is extracted with 0.2% solution of sodium hydroxide.

Now, the total quantity of the proteins thus extracted with the abovementioned solvents was found to be smaller than the total quantity of all the proteins naturally contained in the mulberry leaves. I knew, therefore, that a large amount of proteins remained insoluble in these solvents and that they were retained in the residue.

Then, I treated this residue with 60% boiling alcohol containing 0.3% of sodium hydroxide[1], and thus succeeded in extracting nearly all the proteins retained in the residue, i. e. the protein corresponding in quantity to the greater half of the total quantity of all the proteins naturally contained in the mulberry leaves.

(3) Thus, first estimating the quantities of the proteins by examining the results of the treatments with distilled water → 10% solution of sodium chloride → 70% alcohol → 0.2% solution of sodium hydroxide, in order; and next, treating the residue left after these treatments, by Osborne, Wakeman and Leavenworth's method, i. e. with 60% boiling alcohol containing 0.3% sodium hydroxide, in this way estimating the quantity of the protein thus extracted,—I have proposed, from the view-point of a student of mulberry culture, a method of estimating the comparative quantities of the proteins contained in mulberry leaves.

I also found, after estimating what is generally considered the total quantity of the proteins contained in the mulberry leaves by Stutzer's method, that there always still remained, in the filtrate obtained, no small amount of a protein that could be precipitated by half or complete saturation with ammonium sulphate; so I took the step to precipitate this remaining protein by complete saturation with ammonium sulphate. This precipitate I again dissolved in distilled water; next, by dialysis, I removed the ammonia from this proteinaceous liquid; and then, I estimated the quantity of the nitrogen contained in this liquid.

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* Osborne, Wakeman and Leavenworth. J. of Biol. Chem 49, 63, 1921.

In this way, I also estimated the comparative quantities of the proteose contained in the filtrate which was prepared for the purpose of estimating the total quantity of the proteins contained in the mulberry leaves by Stutzer's method.

(4) Using the same method, I also estimated the comparative quantities of various kinds of protein-nitrogen contained in different parts of the mulberry tree, i. e. its leaves, its stems, its roots, its seeds, and its sap (the white milky juice that oozes out from the leaf-stalks where they are cut off).

Nearly all the proteins contained in the seeds were found to be amenable to the treatments** with water→sodium chloride→alcohol→sodium hydroxide, in order.

With the roots, the results were nealy similar to the results with the seeds.

With the sap, the results were similar to the results with the seeds.

In other words, the proteins contained in the preservatory organs, and the translocating and flowing proteins, were found to be identical with the abovementioned proteins extracted by means of water→sodium chloride→alcohol→ sodium hydroxide, in order.

Among these proteins, globulins predominated; and in the seeds and roots, glutelins came next to globulins, and were also found in great quantity.

On the contrary, the proteins contained in the leaves presented quite a different phenomenon, as has been referred to in (2). In this respect, the stems resembled the leaves more than the roots resembled the leaves, i. e. the total quantity of the proteins that could be extracted by means of water →sodium chloride→alcohol→sodium hydroxide, in order, was found to be about equal to the quantity of the protein that could be extracted from the residue thus obtained, with 60% boiling alcohol containing 0.3% of sodium hydroxide.

Now, considering the results of these experiments to estimate the comparative quantities of the proteins contained in different parts of the mulberry tree by a method proposed from the view-point of a student of mulberry culture, I prepared the following table, which gives in ratios the quantities of protein-nitrogen estimated in various ways, as against 100 of the total quantity of all the protein-nitrogen estimated by Stutzer's method. The figures in this table are based on the results of experiments on fresh materials.

Protein-nitrogen examined in various ways.	Leaves.	Seeds.	Sap.	Stems.*	Roots.
Total quantity of all potein-nitrogen in materials, as estimated by Stutzer's method.	100	100	100	100	100

** The condensed note of the treatments (*) was mentioned on p. 38: and so forth; etc.

Nitrogen precipitated with cupric hydroxide in solution extracted by distilled water.…①	15.12	11.00	88.76	19.94	26.52
Nitrogen precipitated by saturation with magnesium sulphate in solution extracted by distilled water.	12.77	4.25	85.47	13.04	19.49
Nitrógen precipitated with cupric hydroxide but in capable of precipitation with magnesium sulphate in solution extracted by distilled water.	2.35	6.75	3.29	6.90	7.03
Nitrogen extracted with 10% solution of sodium chloride from residue left after treatment with distilled water.……②	7.46	56.00	4.22	18.07	22.15
Nitrogen extracted with 70% alcohol from residue left after treatments with water → sodium chloride.……③	4.41	0.82	0.85	9.00	5.70
Nitrogen extracted with 0.2% solution of sodium hydroxide from residue left after treatments with water → sodium chloride → alcohol.……④	5.01	20.44	1.63	10.40	15.49
Total quantity of nitrogen extracted by four steps above, i. e. ①+②+③+④.	33.39	88.26	95.47	57.43	69.87
Nitrogen extracted with 60% boiling alcohol containing 0.3% sodium hydroxide, from residue left after treatments with water → sodium chloride → alcohol → sodium hydroxide.	62.34	7.60	2.17	42.16	23.38
Nitrogen extracted from residue left after treatment with hot alkaline alcohol, by decomposing said residue with 25% hot hydrochloric acid.	3.26	3.01	1.32	1.11	4.47
Nitrogen contained in residue left after treatment with hot hydrochloric acid.	0.56	0 71	0.31	1.05	1.43

Next, for the purpose of comparing the total quantity of the proteins that could be extracted from different parts of the mulberry tree by means of water → sodium chloride → alcohol → sodium hydroxide, with the quantity of the protein that could be extracted from the residue left after these treatments, by again managing this residue with the hot alkaline alcohol,—I prepared the following table, which gives in percentage the average quantities of these two types of proteins, based on the condensed results of experiments on various materials. The comparisons given in this table are twofold: on the one hand, the results of my experiments on various materials are compared with 100 of the total quantity of the protein-nitrogen contained in these materials, as estimated by Stutzer's method; and on the other hand, the same are compared with 100 consisting of the total quantity of the protein-nitrogen as estimated by Stutzer's method, plus the quantity of proteose-nitrogen contained in the

filtrate left after the experiments by Stutzer's method.

Materials experimented on. \ Methods of estimation	An against 100 of total quantity of protein-nitrogen contained in materials, as estimated by Stutzer's method.		As against 100 consisting of total quantity of protein-nitrogen contained in materials, as estimated by Stutzer's method, plus quantity of proteose-nitrogen contained in filtrate left after experiments by Stutzer's method	
	Total quantity of protein-nitrogen extracted with water → sodium chloride → alcohol → sodium hydroxide	Quantity of protein-nitrogen extracted with hot alkaline alcohol, from residue left after treatments with water → sodium chloride → alcohol → sodium hydroxide	Total quantity of protein-nitrogen extracted with water → sodium chloride → alcohol → sodium hydroxide.	Quantity of protein-nitrogen extracted with hot alkaline alcohol, from residue left after treatments with water → sodium chloride → alcohol → sodium hydroxide
Liaves	33 30	62 34	34 72	61 10
Stems (Average for various stems experimented on.)	47 67	46 58	49 41	45 06
Roots (Average for various roots experimented on.)	60 78	30 35	62.28	29 20
Sap. (i e white milky juice that oozes out from leaf-stalks where they are cut off)	95 47	2 17	95 99	1 92
Seeds.	88 26	7 60	88 55	7 41

(5) After solving with the hot alkaline alcohol the residue of the mulberry leaves that was left after the treatments with water → sodium chloride → alcohol → sodium hydroxide, I also estimated the quantity of the phosphorus contained in this solution. The quantity of the phosphorus was far smaller than even the quantity of the protein contained in the same solution.

Also, after thus solving with the hot alkaline alcohol the residue of the mulberry leaves that was left after the treatments with water → sodium chloride → alcohol → sodium hydroxide, I always noticed that the solution bore a tinge of yellow colour. I am inclined to believe that this was caused by the presence of a flavone-like pigment.

(6) To sum up, a great part of the total quantity of all the proteins containe in mulberry leaves consists of a peculiar proteins, which are retained in the residue left after treatments with water → sodium chloride → alcohol → sodium hydroxide, in order, and are soluble in the hot alkaline alcohol. In other words, the proteins contained in mulberry leaves have many points of difference from the proteins contained in vegetable seeds which have been studied hither to by many workers.

Part II. Concerning the Quantitative Changes of the Proteins Contained in Mulberry Leaves, as Considered with Relation to the Growth of these Leaves.

Résumé.

(1) I made the following studies concerning the quantitative changes of the proteins contained in mulberry leaves, as considered with relation to the growth of these leaves, using the same methods as used in my studies in Part I.

(2) The quantity of each protein contained in the mulberry leaves, calculated in dry leaves, was found to decrease with the growth of the leaves, in proportion as the total quantity of all the proteins contained in the same leaves, calculated in dry leaves, decreased with the growth of the leaves. In other words, if the total quantity of all the proteins contained in the leaves is taken as 100, it was found that the ratios of the total quantities of those proteins that could be extracted with water → sodium chloride → alcohol → sodium hydroxide, in order, fell with the growth of the leaves. But, on the contrary, if the total quantity of all the proteins contained in the leaves is 100, the ratio of the quantity of the protein extracted with the hot alkaline alcohol, from the residue of the leaves left after the treatments with water → sodium chloride → alcohol → sodium hydroxide, in order, was found to increase with the growth of the leaves.

Now, if the total quantity of the proteins extracted with water → sodium chloride → alcohol → sodium hydroxide, in order, is taken as 100, the ratio of the quantity of the protein extracted with the hot alkaline alcohol, from the residue left after the aforementioned treatments, rose considerably with the growth of the leaves, as set forth in the following table :-

	Very young leaves.	Young leaves.	Mature leaves.	Overmature leaves.
Total quantity of protein-nitrogen extracted with water→sodium chloride→alcohol→sodium hydroxide.	100	100	100	100

Quantity of protein-nitrogen extracted with hot alkaline alcohol, from residue left after treatments with water → sodium chloride → alcohol → sodium hydroxide. (As compared with the foregoing.)	442	470	607	721
Total quantity of protein-nitrogen extracted with water→sodium chloride →alcohol→sodium hydroxide, plus quantity of proteose-nitrogen contained in filtrate left after experiments by Stutzer's method	100	100	100	100
Quantity of protein-nitrogen extracted with hot alkaline alcohol, from residue left after treatments with water→sodium chloride→alcohol→sodium hydroxide. (As compared with the foregoing)	360	405	514	614

(3) To sum up, not only the total quantity of the proteins contained in mulberry leaves, calculated in dry leaves, decreased with the growth of these leaves, but also, the ratio of the quantity of the protein extracted with the aid of the hot alcohol from the residue left after the treatments with water→ sodium chloride → alcohol → sodium hydroxide, to the total quantity of the proteins extracted with water → sodium chloride → alcohol → sodium hydroxide, rose considerably with the growth of these leaves. These phenomena show that the proteins contained in mulberry leaves undergo great change in quality as these leaves grow.

Part III. On the Relations in Quantity of the Proteins Contained in the Silkworm-Body and in Silk, to the Proteins Contained in mulberry Leaves, as Examined in Silkworms Fed on mulberry Leaves in Various Stages of Growth.

Résumé.

(1) I stated in Part II that the proteins contained in mulberry leaves undergo great change in quality with the growth of these leaves. That is to say, if the total quantity of all the proteins contained in the leaves is taken as 100, the ratios of the total quantities of those proteins that could be extracted with water → sodium chloride → alcohol → sodium hydroxide, in order, were found to fall with the growth of the leaves; while, on the contrary, the quantity of the protein extracted with the hot alkaline

alcohol from the residue of the leaves left after the treatments with water$\rightarrow$ sodium chloride$\rightarrow$alcohol$\rightarrow$sodium hydroxide, in order (this protein occupies a good half of the total quantity of all the proteins contained in mulberry leaves), were found to increase with the growth of the leaves; also, if the total quantity of the proteins extracted with water$\rightarrow$sodium chloride$\rightarrow$alcohol$\rightarrow$sodium hydroxide, in order, is taken as 100, the ratio of the quantity of the protein extracted with the hot alkaline alcohol, from the residue left after the aforesaid treatments, was found to rise considerably with the growth of the leaves.

Now, here in Part III, I made the following studies concerning the proteins contained in mulberry leaves and the culture of silkworms on mulberry leaves in various stages of growth.

(2) The weight of the silkworm-body, and the ratio of the quantity of the proteins contained in either the fresh or the dry silkworm-body, to the constant weight of the body, were always found to be larger if the silkworm had been fed on young leaves than if fed on mature leaves; and larger again if fed on mature leaves than if fed on over-mature leaves; that is, larger if fed on less matured leaves than if fed on more matured leaves. Consequenty, of the proteins contained in the bodies of a hundred silkworms that had been fed on less matured leaves, was always found to be larger than the absolute quantity of the proteins contained in the bodies of any other hundred silkworms fed on more matured leaves. Also, the weight of the silk-gland of the silkworm, and the ratio of the weight of the silk-gland to the gross weight of the silkworm-body, were found larger if the silkworms had been fed on less matured leaves than if fed on more matured leaves. The weight of the cocoon (as examined in groups of 100 cocoons), and the ratio of the weight of the cocoon-silk to the gross weight of the cocoon $\left(=\frac{\text{weight of cocoon silk}}{\text{gross weight of cocoon}}\times 100\right)$, were found larger in the same way as the foregoing. Conseqently, the absolute weight of the silk taken from a hudred silkworms that had been fed on less matured leaves were found heavier than the absolute weignt of the silk taken fiom any other hundred silkworms fed on more matured leaves.

To sum up, the quantity of the proteins contained in the silkwoam-body and in cocoon silk were always found to increase in proportion to the degree of immaturity of the mulberry leaves used in feeding the silkworms.

The writer of these pages wishes to express his sincere gratitude to Professor Dr. Y. Okuda for his kind advice throughout these works (Part I, Part II and Part III).

Isolation of Citrullin, δ-Carbamido-Ornithin, from the Tryptic Digestion Products of Casein.

By

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(Received February 13, 1933)

Two years ago,[1] the author has isolated a new amino acid, Citrullin, from the pressed juice of water melon, Citrullus vulgaris, Schrad. and proved it to be δ-carbamido-ornithin, represented by the following formula:

$$NH_2\text{-}CO\text{-}NH \cdot \overset{\delta}{C}H_2CH_2CH_2CH(NH_2)COOH.$$

This formula was further confirmed by the synthesis.

Since then, the author has tried to isolate this substance from the hydrolytic products of proteins and studied its behaviour toward acids and alkalies and observed that it is converted to prolin when heated with concentrated acids, while by the action of dilute alkali it is turned into ornithin, carbamino group being splitting off. These facts indicate that citrullin can not exist as such in the acid- or alkaline hydrolytic products, so the author investigated the enzymatic digestion products of proteins. In the first experiment when casein was digested with trypsin in a 0.2% Na-carbonate solution, citrullin could not be isolated and instead of it, ornithin was obtained in fairly good yield. In the next experiment, however, when the digestion was carried out in a neutral solution for 30 days, citrullin was really isolated as copper salt, from which the free acid was regenerated and proved to be identical with the natural product, obtained from water melon. The isolation process was nevertheless very tedious and the yield was far smaller than it was expected. In the third experiment, the method was much simplified and the yield was somewhat better. Still better result is now expected by the tryptic digestion in dilute ammoniacal solution. The details of which will be reported later on.

Experimental

1. Tryptic digestion of casein in a 0.2% sodium carbonate solution.

470 g. commercial casein were rubbed in a mortar with water, neutralised with Na_2CO_3, brought in a large flask, diluted with water to 2 liters and so much anhydrous sodium carbonate was added until it reaches 0.2% of the liquid and after adding 2.5 g. trypsin (Merck) and 10 ccm. toluol, the flask was kept at 37° with frequent shaking. From time to time the reaction was tested with congo paper and so much sodium carbonate was added until the paper turned red. After 30 days when the solution became yellowish

brown and white crystalline cluster deposited at the bottom, it was filtered, neutralized with sulphuric acid, evaporated to 500 ccm., acidified with sulphuric acid and treated with phosphotungstic acid. The precipitate formed thereby was collected by suction, dissolved in 50% acetone, decomposed with baryta in. usual way and the filtrate containing free bases was treated with 30% silver nitrate and baryta until no more precipitate was formed, filtered off and the filtrate i.e. the lysin fraction was freed from silver and baryta by sulphuric acid and hydrogen sulphide respectively, evaporated to 400 ccm, acidified with sulphuric acid and once more precipitated with phosphotungstic acid. The free bases regenerated from this precipitate were converted into picrates, which were revealed to be the mixture of ornithin and lysin. By treating the above picrates with methyl alcohol, ornithin picrate was dissolved leaving lysin picrate as insoluble residue The methyl alcoholic extract was now evaporated in vacuum, again treated with methyl alcohol to remove the little insoluble residue. After repeating this operation five times 49.9 g. ornithin picrate (ca. 3% of casein used) were obtained. It was converted into copper salt and analysed:

7.198 mg. subst. 1.041 ccm. N_2(757.6 m.m. 14°) = 16.88% N
Calculated for ornithin copper = 17.19% N

2. Tryptic digestion of casein in neutral solution.

500 g. commercial casein were rubbed in a mortar with water, adding sodium carbonate until neutral reaction was attained, the thick paste thus obtaind was brought in a large flask, diluted with 6 liters water and after adding 5 g. trypsin (Merck) and 10 ccm. toluol, the flask was kept at 37°, shaking from time to time. The acidity developed during the digestion was carefully neutralized with sodium carbonate. After two weeks the reaction became constant, so that the neutralization was no more necessary and greyish white crystalline cluster, consisting chiefly of tyrosin began to deposit at the bottom. After 30 days it was filtered and the yellowish brown filtrate was concentrated in vacuum to a small volume and treated with five times of its volume of methyl alcohol. The precipitate formed thereby was collected after 24 hours, dissolved in water and fractionally precipitated with silver nitrate and baryta. The last fraction obtained by adding an excess of baryta, consisted of a reddish brown precipitate giving the typical reaction of citrullin. It was treated with sulphuric acid and then with hydrogen sulphide to remove the baryta and silver completely, and after expelling off the hydrogen sulphide, the citrullin was isolated as the copper salt. The yield was 1.05 g. The free citrullin regenerated from the copper salt forms colourless long thin prisms, melting at 226°. In every respect it was identical with the natural product. It was dried at 100° and analysed:

5.648 mg. subst. 1.225 ccm. N_2(757.5 m.m. 28°)=23.64% N

3.786 mg. subst. 5.661 mg. CO_2=40.78% C 2.595 mg. H_2O=7.62% H

Found	C% 40.78	H% 7.62	N% 23.64
Calc. for $C_6H_{13}N_3O_3$	41.11	7.48	23.99

The free base was again converted into copper salt and analysed:

5.761 mg. subst. 1.008 ccm. N_2(757,6 m.m. 14.5°)=20.36% N

4.428 mg. subst. 5.533 mg. CO_2=34.07% C 2,339 mg. H_2O=5.86% H.

Found	C% 34.07	H% 5.86	N% 20.36
Calc. for $(C_6H_{12}N_3O_3)_2Cu$	34.79	5.84	20.31

When citrullin was precipitated with silver nitrate and baryta as described in the above experiment and the precipitate was directly boiled with 5 ccm. hydrogen iodide for 8 hours, citrullin was converted into prolin. For the isolation of the latter, the boiled mixture was diluted with water, filtered and evaporated to expell off the HI, boiled with copper hydroxide, filtered, evaporated and treated with methyl alcohol. The copper salt of prolin obtained in this way was purified by recrystallisation, dried at 100° and analysed:

5.184 mg. subst. 0.438 ccm. N_2(759.3 m.m. 17°)=9.75% N

Calc. for prolin copper =9.87% N

3. Formation of prolin from citrullin.

The formation of prolin from citrullin by the action of strong acids is demonstrated in the following experiment:

0.5 g. citrullin, isolated from water melon was boiled with three times of conc. HCl for 8 hours under reflux cooler. After distilling off the hydrochloric acid in vacuum, the residue was dissolved in a little water and boiled with $Cu(OH)_2$. The filtrate therefrom was evaporated and treated with ethyl alcohol, the soluble cupric chloride was filtered off, and the precipitate was washed with ethyl alcohol. It was easily soluble in water, but almost insoluble in methyl alcohol. In this way, the copper salt of prolin was obtained. After recrystallization, it was dried at 100° and analysed:

3.030 mg. subst. 0.270 ccm. N_2(759,3 m.m. 26°)=9.89% N

Calc. for prolin copper $(C_5H_8NO_2)_2Cu$ =9.87% N

From the copper salt, free prolin was prepared. It was dissolved in ethyl alcohol and precipitated with ether, the precipitate thus obtained was again extracted with butyl alcohol, once more recrystallized from ethyl alcoholic solution by adding ether, dried at 100° and analysed:

5.961 mg. subst. 0.659 ccm. N_2(754.8 m.m. 25°)=11.90% N*

Calc. for prolin $C_5H_9NO_2$ =12.18% N

Heated in a capillary it begins to melt at 200° and decomposes at 220°: By fusion the characteristic odour of pyrrolidin is developed.

The author has further observed that ornithin is very resistant toward strong acids. Even by prolonged heating with conc. hydrochloric acid only a portion of it is converted into prolin. Further, the fact that ornithin is never formed by the acid hydrolysis of proteins indicates that it is not the primary decomposition products of proteins, so it is self-evident that prolin can not be derived from ornithin.

As prolin is rather easily formed from citrulin by the action of acid it is more probable that during the acid hydrolysís, at least a portion of prolin is derived from citrullin, though it may also be formed as the primary product.

Ornithin may be formed secondarily either from arginin or from citrullin during the alkaline hydrolysis or during the tryptic digestion of proteins. The author has further estimated the distribution of different amino acids according to the method of van Slyke in the acid and alkali hydrolytic products of proteins and found that the nonamino-N, i.e. the prolin fraction is always higher in the acid hydrolysis. This might be due to the formation of prolin, at least partly, from citrullin.

D. Ackermann[2] has recently reported on the formation of citrullin from arginin by the putrifying process. In the experiment of the present auther however no bacterial growth was observed during the tryptic digestion of casein, so it is evident that microorganisms has no casual relation with the formation of citrullin from proteins, but the question whether citrullin might be formed from arginin by the action of trypsin, remains to be settled. For such a reason the author carried out the following experiment:

2.752 g. arginin carbonate were dissolved in 27 ccm. water to which 0.1 g. trypsin (Merck) was added besides a little chloroform and toluol. After standing for 6 weeks at 37°, the solution was filtered and treated with 5.5 g. flavianic acid and a few drops of conc. HCl and after warming for a short time, it was left for 24 hours when nice plate crystals of arginin flavianate separated out, which were collected by suction, washed with ethyl alcohol and dried in a vacuum desiccater. In this way 5.95 g. arginin flavianate, corresponding to 2.752 g. arginin carbonate were recoverd. As no citrullin could be detected in this case, it is evident that trypsin had no action upon arginin.

Literature

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Über Prolysin, α-Amino-ε-hydantoincapronsäure, ein neues Abbauprodukt des Eiweisses. I.

Von

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Vor Kurzem, als der Verfasser sich mit der Isolierung des Citrullins aus tryptischen Verdauungsprodukten des Caseins beschaftigte, beobachtete er, dass der Verdauungssaft ausser Citrullin noch eine Substanz enthalt, welche die für Harnstoffgruppen charakteristische Schiffsche sowie P. Ehrlichsche Reaktion gibt. Diese Substanz ist gegen Sauren bestandiger als Citrullin und existiert noch nach dem Zerstoren des Citrullins durch verdünnte Salzsaure in die Flüssigkeit. Sie entsteht auch bei vorsichtiger Verdauung des Eiweisses durch verd. Säuren. Durch langeres Erhitzen mit konz. Sauren order Alkalien wird sie aber vollstandig zerstort. Am besten kann man eine alkoholische Salzsaurelosung fur die Verdauung anwenden.

Seitdem hat der Verfasser sich bemüht, diese Substanz zu isolieren. Wenn die Verdauungsflüssigkeit, die obengenannten Reaktionen gibt, bis zu schwach saurer Reaktion neutralisiert, mit Tierkohle entfarbt und mit einer verd. alkoholischen Losung von Xanthydrol versetzt wird, so kann man diese Substanz als Xanthylverbindung in farblosen Prismen bekommen. Ferner, wenn die neutralisierte Verdauungsflüssigkeit nach starkem Einengen im Vakuum mit fünffacher Menge Methylalkohol versetzt wird, so entsteht ein Niederschlag, der starke Schiffsche Reaktion gibt. Wird nun dieser Niederschlag in Wasser gelost und mit Silbernitrat und Baryt fraktioniert gefallt, so erhalt man diese Substanz als Silbersalz, welches in üblicher Weise in kristallinisches Kupfersalz verwandelt und gereinigt wird.

Die aus reinem Kupfersalz regenerierte freie Substanz bildet ein hygroskopisches, weisses, kristallinisches Pulver, das loslich in Wasser, unloslich in Alkohol und Aether ist. Es gibt die Schiffsche, P. Ehrlichsche Jaffesche sowie die Biuret und Triketohydrinden Reaktionen; es bildet hellblaues Kupfersalz wie gewohnliche Aminosauren und auch kristallinisches Pikrat. Gegen Xanthydrol verhalt es sich wie Harnstoff und bildet kristallinische Monoxanthylverbindung.

Die Analyse der freien Substanz stimmt mit der Formel $C_8H_{13}N_3O_4$ überein.

Beim Kochen mit konz. Sauren oder Alkalien wird sie glatt in Lysin gespalten. Wahrscheinlich bietet sie eine Vorstufe des Lysins in Eiweissmolekül

dar. So hat der Verfasser den Namen "*Prolysin*" für diese Substanz vorgeschlagen.

Durch Reduktion und Hydrolyse des Prolysins mit Jodwasserstoff bildet sie α-Monoaminopimelinsaure, die weiter in Pimelinsaure gespalten wird.

Durch Einwirkung von Bariumhydroxyd bei Zimmertemperatur wird zuerst α-Uramino-α'-aminopimelinsaure gebildet, welche weiter in Diaminopimelinsaure verwandelt wird.

Schwefelwasserstoff und Bariumcarbonat spalten sie in α-Uramino-α'-aminopimelinsaure und weiter in Harnstoff und α-Aminopimelinsaure.

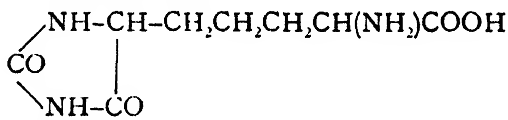
Durch Salpetersaure bei gewohnlicher Temperatur oxydiert, wird Parabansaure gebildet.

Mit Harnstoff kondensieit, bildet sie Pentamethylendihydantoin, die durch Erhitzen mit Bariumhydroxyd in Cadaverin verwandelt wird.

α-Aminopimelinsauie liefert in analoger Weise mit Harnstoff ε-Hydantoincapronsaure, die beim Kochen mit Bariumhydroxyd wieder in ε-Aminocapronsaure gespalten wird.

Durch Jodierung des Prolysins werden zwei Wasserstoffatome der Imidoradikales substituiert.

Aus den oben erwahnten Beobachtungen hat der Verfasser dem Prolysin die folgende Formel zugeschrieben:

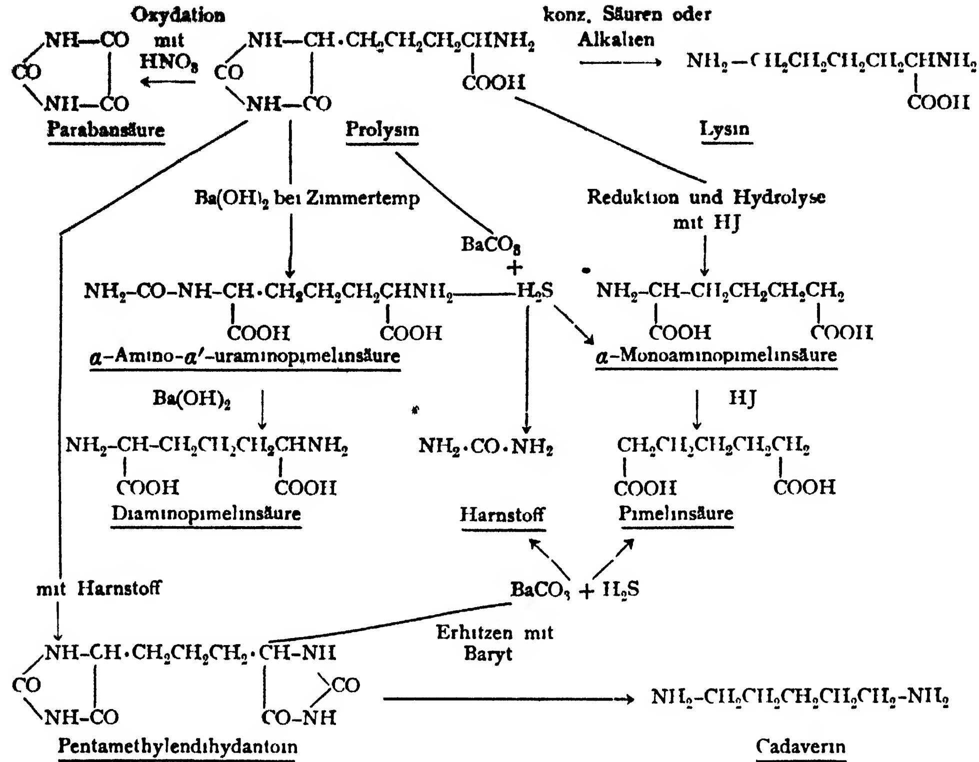


Prolysin: α-Amino-ε-hydantoincapronsaure.

Umwandlung und Spaltungsweise des Prolysins werden in der folgender Seite schematisch dargestellt.

Isolierung des Prolysins

900 g Gelatine wurden mit 900 ccm salzsauiegesattigtem, 60%igem Alkohol 8 Stunden gekocht (die Temperatur der Flüssigkeit war etwa 90°). Das Hydrolysat zeigte starke Biuretreaktion sowie die Schiffsche Reaktion (rotviolette Farbung mit Furfurol, Aceton und konz. Salzsaure). Es wuide mit 300 ccm 50%iger alkoholischer Natronlauge neutralisiert, eingedampft, filtriert und mit 50%igem Alkohol gewaschen. Das Filtrat wurde dann im Vakuum zum Sirup eingedampft und mit funffacher Menge Methylalkohol versetzt, wodurch ein Niederschlag entstand, welcher starke Schiffsche sowie P. Ehrlichsche Reaktion gab. Der Niederschlag wurde in Wasser gelost und mit Silbernitrat und Baryt fraktioniert gefallt. Da die in saurer Losung gebildete gelbbraune Fallung kein Prolysin enthielt, wurde sie abfiltriert und zum Filtrat soviel Baryt zugegeben bis es pH 5.8 erreichte. Das Prolysin wurde dadurch fast



vollstandig als hellgelber amorpher Niederschlag gefallt. Nach einem Tag wurde es abfiltriert, sukzessiv mit wenig Wasser, Methylalkohol und Aether gewaschen und im vakuum über Schwefelsaure getrocknet. Die Ausbeute des Silbersalzes betrug etwa 12 g. Um freies Prolysin darzustellen, wurde das Silbersalz in 50%igem Alkohol suspendiert, durch Schwefelsaure und Schwefelwasserstoff von Baryt bezw. Silber befreit, eingedampft und mit Methyalkohol und Aether behandelt. In der Weise erhielt man das Prolysin als weisses, kristallinisches Pulver. Es bildet hellblaues kristallinisches Kupfersalz vom Schmelzp. 260°, welches in Wasser sehr leicht, in Methyl- und Aethylalkohol aber unloslich ist. Das Pikrat des Prolysins kristallisiert in gelben Plattchen, schmilzt bei 122°. Es ist in Wasser leicht, in Methylalkohol und Aether unlöslich. Das reine freie Prolysin, regeneriert aus reinem Kupfersalze, ist ein weisses, kristallinisches Pulver, loslich in Wasser, unlöslich in Methyl- Aethyl- Butylalkohol, Aceton, Aether usw. Im Kapillarrohr erhitzt, beginnt es bei 209° sich zu farben und zersetzt es sich bei 222°.

Die wasserige Lösung reagiert neutral gegen Lakmus. Aus konzentrierter Lösung wird es durch Phosphorwolframsaure gefallt, nicht aber aus verdünnter Lösung. Aus alkalischer Lösung wird es durch Quecksilbersalze,

(wie Acetat, Nitrat, Sulfat und Chlorid) gefallt. Silbernitrat gibt in einer Lösung von pH 5.6 bis 6.8 durch Zusatz von Baryt einen hellgelben Niederschlag. In saurer oder ammoniakalischer Losung wird aber keine Fallung hervorgerufen. Das Silbersalz ist schwer loslich in Wasser und wird durch Zusatz von Alkohol wieder gefallt. Es lost sich in verdünnter Salpetersaure (6 bis 7%) und fallt wieder durch Zusatz von Baryt aus (bei pH etwa 6). Durch dieses Verfahren wird es am besten gereinigt. Phosphormolybdansaure oder basisches Bleiacetat geben keine Fallung. Urease hat auch keine Wirkung auf Prolysin.

Farbenreaktionen des Prolysins

(1) Triketohydrindenreaktion: violett.

(2) Biuretreaktion: stark. Allantoin und Harnsaure geben auch dieselbe Reaktion.

(3) P. Ehrlichsche Reaktion (p-Dimethylaminobenzaldehyd und Salzsaure): gelblichgrün.

(4) Schiffsche Reaktion (Furfurol, Aceton und konz. Salzsaure): violettrot. Allantoin und Citrullin geben auch dieselbe Reaktion

(5) Phenol und Natriumhypochlorit: blau.

(6) Adamkiewiczsche Reaktion (konz. Schwefelsaure und Pepton): rotviolett. Allantoin gibt wieder dieselbe Reaktion.

(7) Jaffesche Anhydridreaktion (Erhitzen mit Pikrinsaure und Natriumcarbonat): rot. Bei Allantoin dieselbe Reaktion.

(8) 1% Pyrogallol in konz. Schwefelsaure: schon rotviolett. Allantoin: gelblichgrün.

(9) 0,1% Indollosung und konz. Schwefelsaure: rot.

(10) 0,1% Skatollosung und konz. Schwefelsaure: gelblichrot.

(11) Paulysche Diazoreaktion: orange.

(12) Fehlingsche Losung: beim längeren Erhitzen reduziert.

(13) Ferriferricyanidreagens: grünlichblau.

(14) Pyrrolreaktion: stark.

(15) Ferrichlorid: rote Fallung.

(16) Die folgenden Reaktionen fallen negative aus: Millon, Sakaguchi, Murexid, Folin, Morner und Liebermannsche Nitrosoprobe.

Analyse des freien Prolysins:

4,946 mg Substanz 8,043 mg CO_2, 2,955 mg H_2O,
6,723 mg Substanz 1,108 ccm N_2 (758,1 mm 17°)

	C	H	N
Gef.	44,34	6,63	19,33
Ber. $C_6H_{13}N_3O_4$	44,62	6,09	19,53

Analyse des Kupfersalzes:

3,383 mg Substanz 4,861 mg CO_2, 1,523 mg H_2O,
3,505 mg " 0,508 ccm N_2 (759,8 mm 16°),
3,383 mg " 0,554 mg CuO

	C	H	N	Cu
Gef.	39,19	5,00	17,11	12,97
Ber. $(C_8H_{12}N_3O_4)_2Cu$	39,03	4,91	17,08	12,92

Monoxanthylprolysin 1 265

Prolysinpikrat 1 51

α-Monoaminopimelinsäure 1 165
(Kupfersalz)

On the so-called "Imo-shōchû" the Sweet potato spirit by Old Method.

By

Kotaro NISHIDA

(*Kagoshima Agricultural College, Kagoshima, Japan.*)

(Received February 28, 1933.)

The so-called "Imo-Shōchû" is a kind of spirit obtained from "Imo" (Sweet potato) and "koji" by old method. It is a special product of two prefectures—Kagoshima and Miyazaki—in our country. The annual production of this spirit in these districts amounts to about 100,000 hectolitres.

As its raw materials there are used (1) steamed sweet potato, (2) 'Awamori koji" or black koji, a steamed foreigen rice upon which *Aspergillus Awamori* has been developed, and (3) water.

The spirit mash is prepared by mixing these raw materials in following proportion.

	In the 1st mixing	In the 2nd mixing	Total	Riped mash
Sweet potato	– –	450 kg	450 kg	785 litres
"Awamori koji" rice	130 litres	——	130 litres	
water	145 litres	247 litres	392 litres	

In a large earthen pot, "Awamori koji" prepared from 130 litres of foreign rice, is allowed to stand in 145 litres of water for about a week. Then the second mixing materials is added to the first mixture, and after about 10 days, the mash is distilled by very simple distillator. The yield of the sweet potato spirit, containing 40% alcohol by volume, is about 244 litres from 785 litres of riped mash.

Experimental Results.

I. Chemical Composition of "Awamori koji" and its Extract.

The analytical results of "Awamori koji" and its saccharified extract (Balling=10°, PH=3.11), are shown in the following table:

	In 100 g of "Awamori koji" (g)	In 100 c.c. of koji extract (g)
Moisture	21.79	90.31
Solid matter	78.21	9.69
Total N	1.1062	0.1274
Protein N	0.9544	0.0038

Non-protein N	0.1518	0.1236
Ether extract	0.2916	——
Alcohol extract (free from ether extract)	1.9883	——
Reducing sugar (as glucose)	1.8537	7.1076
Starch and Dextrin	62.13	——
Total carbohydrate (as glucose)	——	8.9223
Dextrin	——	1.6332
Total acid (as citric acid)	——	0.7749
Ash	0.4859	0.1035

II. Chemical Changes of Mash during its Saccharifying and Fermenting.

In the spirit mash, as in the case of "Saké", the saccharification and the alcoholic fermentation takes place at the same time, but in the former case, these actions arise suddenly.

The analysis of mash is carried on 17 days during its ripening with the following results:

Date	Temp. of mash	PH	g in 100 c.c filtrate of mash		
			Acid (as citric acid)	Reducing sugar (as glucose)	Alcohol
1 (Jan. 11)	25°C	2.96	0.9645	2.2653	0 05
2 (〃 12)	24	2 87	1.6529	3.2020	3.46
3 (〃 13)	28	3.12	1.4866	0.4646	9.85
4 (〃 14)	27	3.24	1.4700	0.7630	11.72
5 (〃 15)	23	3 29	1.4434	0.8173	13.28
6 (〃 16)	17	3.36	1.4367	0.7841	14 00
7*(〃 17)	22	3.96	0.3559	1.7142	7 62
8 (〃 18)	30	4.07	0.2860	1.0295	8.46
9 (〃 19)	29	4.21	0.2661	0.4562	10.90
10 (〃 20)	25	4 26	0.2494	0.3269	11.49
11 (〃 21)	24	4.29	0.2594	0.2890	11.66
12 (〃 22)	22	4.26	0.2561	0.2798	11.52
13 (〃 23)	20	4.03	0.3193	0.3090	11.02
14 (〃 24)	18	4.09	0.2927	0.2731	11.08
15 (〃 25)	18	4.07	0.3093	0.2731	11.14
16 (〃 26)	16	4.12	0.2993	0.2731	11.14
17 (〃 27)	14	4.05	0.2993	0.2731	11.08

* Shows the second mixing of the raw materials.

III. Composition of Sweet potato spirit and its Byproduct.

General composition of the spirit prepared by above method, is as follows:

Specific gravity	0.9504	PH	6.01
Alcohol (vol. %)	40.38	Extract (g in 100 c.c.)	0.0317
Ash (g in 100 c.c.)	0.0182		

The distillated residue obtained from the mash, is used as fodder especially for pig, or manure; its analytical data are shown in the following table:

	g in 100 c.c. of original sample	In 100 parts of dry matter
Water	95.10	——
Dry matter	4.90	——
Crude protein	0.9402	19.19
Protein	0 7050	14 39
Crude fat	0 3123	6.37
Crude fibre	0.4664	9.52
Crude ash	0.4055	8.28
Extract	2 9000	59.18
Nitrogen free extract	2 7756	56.64

Summary.

(1) The titration acidity and hydrogen ion concentration of the "Awamori-koji" extract are very higher than those of the "Saké-koji" extract, that is:

	PH	g in 100 c.c.	
		as citric acid	as lactic acid
"Awamori-koji" extract	3.11	0.7749	——
"Saké" extract	5 49	——	0 0795

(2) The changes of the spirit mash during its ripening, are summarized as follows:

(a) The temperature of the mash is very high and the maximum reaches to 30°C or above.

(b) The titration acidity of the mash is very large, using the "Awamori-koji" as raw material. The organic acid in the mash is mainly citric, and moreover succinic acid.

(c) The hydrogen ion concentration of the mash is remarkably large, namely its PH value is below 3.0 at the beginning of the mash; therefore the bacteria could not multiply in these medium even in a hot district. But by the second mixing of raw materials (sweet potato and water) the PH value of mash is elevated suddenly, on account of the phosphates in the sweet potato. In this time of the mash, the resinous substances in sweet potato played as an antiseptic. Thus the fermentation of mash may be carried out very safely.

(d) The amount of reducing sugar in the mash is very small, while on

the other hand the formation of alcohol is very active. From these facts, it may be said that, the sugar formed from the starch was changed to the alcohol immediately in succession.

(e) In the spirit mash a small amount of alcohol is lost nearly at the end of fermentation of mash by the evaporation. Then the distillation of mash should be performed at about the 5th day after the 2nd mixing of raw materials, when the content of alcohol reaches to the maximum.

Über Säuren und Alkohols im hochsiedenden Anteil des Fuselöls

Von

T. Taira

(Eingegangen am 3. März 1933)

Bekanntlich ist das Fuselol ein Nebenprodukt der alkoholischen Garung, und wegen seiner Wichtigkeit für die Garungschemie wie auch fur die Hygiene ist es der Gegenstand zahlreicher Untersuchungen gewesen. Verhaltnismassig gering ist aber die Anzahl der Arbeiten über die hoher als Amylalkohol siedenden Anteile. Zwar wird angegeben, dass darin Fettsauren wie Capron–, Oenanthyl–, Capryl–, Pelargon–, Laurin–, Myristin– und Palmitinsaure, ferner Alkohole wie n–Hexyl–, Isohexyl–, n–Heptyl–, Oktyl–, Nonyl– und Decylalkohol sowie Terpineol vorkommen, aber da bei bisherigen Arbeiten geringe Mengen Material verarbeitet und nur indirekte Methoden z. B. Elementaranalyse der betreffenden Fraktionen, Molekulargewichtsbestimmung und Analysen der Salze zum Nachweise benutzt wurden, so ist nach Ansicht des Verf. die Untersuchung durch direkte methoden sehr erwunscht.

Dem Verf. standen relativ grosse Mengen Material bei der Untersuchung des Fuselols aus Rohrzucker–Melasse, Ipomoea und Kaoliang (in China einheimische Art Mohrenhirse der Familie Graminae) und des Reisbranntweins (rice brandy oil) zur Verfugung. Nachdem unter gewohnlichem Druck die unterhalb 132°C siedenden Anteile abdestilliert worden waren, wurde der Rückstand verseift, um durch Extraktion desselben mit Äther die Seife vom unverseifbarem Anteil zu trennen. Nach Zersetzung der Seifenlösung mit Mineralsaure wurden die Fettsäuren mit Äther extrahiert. Die atherische Lösung wurde dann mit geglühtem Natriumsulfat getrocknet und der Äther abdes-

tilliert Die Fraktionierung des Rückstands wurde unter Benutzung des Widmer'schen Kolonnenapparats vorgenommen. Aus dem unter 10 mm bis 200°C übergehenden Anteile von einheitlichem Molekulargewicht wurden die Anilide hergestellt, um deren Schmelzpunkte zu bestimmen, ferner durch Mischprobe den Identitätsnachweis zu erbringen. Der unter 10 mm über 200° übergehende Anteil wurde nach dem Äther-Bleisalzverfahren behandelt, um die gesättigten von den ungesättigten Säuren zu trennen. Die gesättigten Säuren wurden dann einer fraktionierten Kristallisation aus Alkohol unterworfen, um schliesslich deren Schmelzpunkte und Molekulargewichte zu bestimmen. Von den ungesättigten Säuren wurden Bromadditionsprodukte hergestellt und so Hexa-, Tetra- und Dibromide erhalten und auf diesem Wege die einzelne ungesättigte Säure nachgewiesen. Die ätherische Lösung des unverseifbaren Produkts wurde mit geglühtem Natriumsulfat getrocknet, dann der Äther verjagt und der Rückstand unter Benutzung des Widmer'schen Kolonnenapparats fraktioniert. Jede Fraktion wurde noch einmal fraktioniert und so gereinigt. Dann wurden die physikalischen Konstanten jeder gereinigten Fraktion bestimmt. Sodann wurden die Fraktionen mit Chromsäuregemisch oxydiert und Semicarbazone aus den Oxydationprodukten gewonnen. Phenyläthylalkohol wurde durch Phenylurethan charakterisiert.

Tabell I.

(T. Taira, Journ. Agr. Chem. Japan IX, 7~18, 1933.)

	Fuselöl aus Rohrzuckermelasse	Ipomoea-Fuselöl	Kaoliang Fuselöl	Rice brandy oil
Capronsäure	4.7%	2.9%	+	(5)
Caprysäure	19.8	6.7	?	(2)
Caprinsäure	45.8	10.0	12.0%	(1)
Laurinsäure	5.3	7.2	?	(4)
Myristinsäure	0	0	?	(3)
Palmitinsäure	14.7	42.3	32.0	63.0%(roh)
Ölsäure	7.9	24.0		22.0
Leinölsäure	1.5	7.2	28.0	9.0
Linolensäure	0.3	0		0

Anmerkung: Die Klammern in der Rubrik "Rice brandy oil" der obigen Tabelle die Reihenfolge der Mengen. Beim Kaoliang-Fuselöl und Rice brand oil-Fuselöl ist der Gehalt der Myristinsäure sehr gering, sodass befriedigende Resultate nicht erhalten werden konnten.

Tabelle. II.

(T. Taira Joun. Agr. Chem. Japan, 1933, im Druck befindlich.)

	Methyl-n-amyl-carbinol	Methyl-n-heptyl-carbinol	Phenyläthyl-alkohol
Fuselöl aus Rohrzuckermelasse (36 L)	30 g	40 g	2 g
Ipomoea-Fuselöl (9 L)	2	3	16
Rice brandy oil (30 L)	ca. 2.0 g		6
Saké (27 L)	ca. 0.7 g		1

Von den bisher durch verschiedene Forscher als Bestandteile des Fuselöls angegebenen zahlreichen Fettsäuren können gesättigte Fettsäuren mit gerader Kohlenstoffzahl C_6 bis C_{16}, ferner die vom Verf. zuerst darin nachgewiesenen ungesättigten Fettsäuren mit gerader Kohlenstoffzahl, nämlich Ölsäure und Leinölsäure, als regelmässige Bestandteile des Fuselöls bestätigt werden. Aber Fuselöl aus Rohrzucker-Melasse und Ipomoea-Fuselöl enthalten keine Myristinsäure. Ferner enthält das Fuselöl aus Rohrzuckermelasse gering Mengen Linolensäure. Oenanthylsäure und Pelargonsäure, die bisher öfters als Bestandteile des Fuselöls angegeben wurden und die Fettsäuren mit ungerader Kohlenstoffzahl sind, konnten in keinem Fuselöl gefunden werden.

Die bisher im Fuselöl vermutenen primaren normalen Alkohole und Alkohole der Isoform über C_6 sind auch nicht im unverseifbaren Teil enthalten. Sehr interessant ist die Tatsache, dass sekundäre Alkohole mit C_7 und C_9 darin vorkommen. Zwar fehlen in der Literatur Angaben, dass Phenyläthylalkohol, ein Garungsprodukt des Phenylalanins, im Fuselöl vorkommt, doch ist dieser Alkohol im Ipomoea-Fuselöl, im Fuselöl des rice brandy oil und im Saké deutlich nachweisbar enthalten.

Dass sekundäre Alkohol des Fuselöls durch Phytoreduktion der durch β-Oxydation der Fettsäuren mit gerader Kohlenstoffzahl entstandenen Methylketone sich bilden, scheint wahrscheinlicher zu sein als die Ehrlich'sche Theorie einer Bildung durch Gärung der Aminosäure. Arbeiten über β-Oxydation durch Schimmel pilze sind zwar bekannt, aber letztere wurden nicht bei der Hefeentdeckt. Der Umstand, dass im Fuselöl aus Rohrzucker-Melasse, in welchem Fall Hyphomyceten nicht zur Wirkung kommen können, doch sekundäre Alkohole enthalten sind, scheint dafür zu sprechen, dass diese Wirkung der Hefe zukommt.

K. Shoji hat fast zur gleichen Zeit wie Verf. mitgeteilt, dass er aus rice brandy oil Methyl-n-amylcarbinol und Methylheptylcarbinol isolieren konnte.

(Scientific Papers of the Institute of Physical and Chemical Reserch. vol. 20 (1933) no. 405)

Nutritive Value of Sperm Whale Oil and Finback Whale Oil.

By Yoshikazu SAHASHI.

(Received February 10, 1933.)

Though nutritional studies of fats or oils have already been carried out by many investigators, little is yet attempted in the feeding experiments with such oils as sperm whale oil which contains a large amount of waxes.

The present author, therefore, carried some feeding experiments of rats with synthetic diets containing large amounts of the oils obtained from sperm whale (*Physeter macrocephalus* L.) and finback whale (*Balaenoptera physalus* L.) for the purpose of contributing something to this nutrition problem.

Through the previous work of M. Tsujimoto[1] and Y. Toyama[2], the chemical properties of the oils prepared from sperm whale and finback whale have already been determined as follows:

	The head oil from sperm whale (M. Tsujimoto)	The body oil from sperm whale (M. Tsujimoto)	Finback whale oil (Y. Toyama)
Sp. gr.	$d_4^{15} = 0.8848$	$d_4^{20} = 0.8806$	$d_4^{15} = 0.9231$
Refr. index	$n_D^{20} = 1.4633$	$n_D^{30} = 1.4620$	$n_D^{40} = 1.4727$
Acid value	0.99	1.24	2.21
Saponification value	147.1	131.6	196.6
Iodine value	71.4	82.4	112.9
Reichert-Meissel value	0.57	——	——
Unsaponifiable matter	36.0%	36.4%	1.09%
Fatty acids	65.0%	64.13%	——
Glycerol	3.52%	——	——
Cholesterol	0.18%		

The sperm whale oils consisted chiefly of mixed waxes. The unsaponifiable matter (wax alcohols) contains cetyl alcohol and oleic alcohol in nearly equal proportion. Small amounts of octadecyl and tetradecyl alcohol appear to be present. The total percentage of cholesterol was found to be 0.18% of the sperm oil. The fatty acids consisted of about 19% solid and about 81% of liquid acids. Among the solid (saturated) acids palmitic and miristic have been identified. A small quantity of caprylic or capric acids was also present. The liquid (unsaturated) acid consisted mainly of the oleic acids series: physetoleic and oleic acids. Not more than 1% of highly unsaturated acids was also present. On the contrary, the finback whale oils contained fatty acids of the same composition which occur in other animal or vegetable

oils as common glycerides.

The present author carried out feeding experiments with a genuine head oil and an intestine oil obtained from sperm whale (male: body length about 14 meters) and with a blubber oil and an intestine oil prepared from a finback whale. These samples were kindly supplied from Tōyō Hogei Kaisha.

Experimental.

(1) *Experiments with diets containing whale oils in sufficient quantity.*

The analysis of the samples used in the experiments:

	Sperm whale		Finback whale	
	The head oil	The intestine oil	The blubber oil	The intestine oil
Sp. gr.	——	——	$d_4^{15} = 0.916$	$d_4^{15} = 0.916$
Refr. index	——	$n_D^{21} = 1.464$	$n_D^{22} = 1.466$	$n_D^{22} = 1.467$
Acid value	0.9	2.0	0.2	0.9
Sap. value	145.4	144.4	185.5	180.5
Iod. value	62.7	88.6	92.7	97.6
Vitamin A	——	——	——	——

For the experiments: Albino rats weighing 40～50 g each, were previously fed for several days on a complete diet until they reached 50～60 g. Then they were divided into several groups, each consisting of four animals, and were fed on various experimental synthetic diets. (Fig. 1～8)

The diets consisted of:

Potato starch (Japanese Pharmacopoea IV)	65 g	60 g
Fish meat protein (bonito meat freed from fat)	15″	15″
McCollum's salt mixture	4″	4″
Oryzanin solution (Sankyo & Co.)	5 cc	——
Dried yeast (supplied from Oriental Company extracted with ether)	——	2″
Whale oils	**15 g**	**20 g**
Biosterin, dissolved in olive oil, given per os daily.	1 mg	1 mg

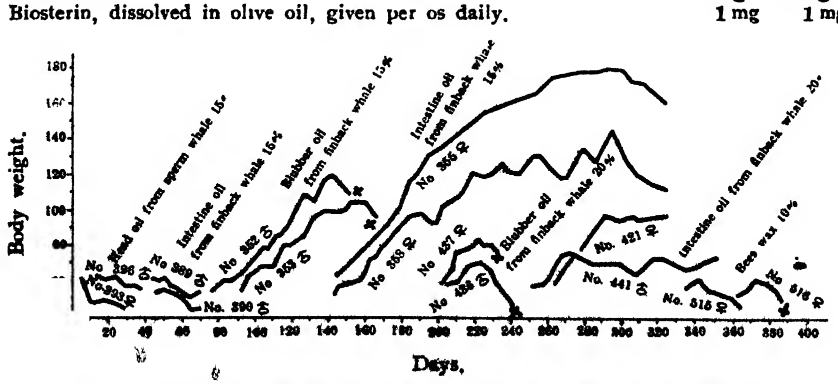


Fig. 1. The growth curves of the rats fed on diets containing whale oils.

(II) *Experiments with waxes and purified sperm oils.*

In order to investigate the characteristic symptom of rats fed on the diets containing sperm oils as described above, the author attempted to purify the waxes from sperm oils.

i) Spermaceti (cetin): When the head oil from sperm whale was cooled at 0~5°C, a large quantity of the solid wax which consisted chiefly of cetin separated at the bottom as white crystalline mass. It was collected, freed from the mother liquor (so-called winter sperm oil) by centrifugal machine, and recrystallized from hot alcohol.

ii) Liquid waxes: 100 g said winter sperm oils freed from the above solid waxes were boiled with 400 c.c. of a 3% Na_2CO_3 solution for 3 hours. The impurities in the oils are thus completely dissolved. After cooling, the liquid oils were separated and washed with water until the wash water contained no trace of sodium carbonate. Analysis of waxes:

	Solid waxes	Liquid waxes		
	Spermaceti (cetin) (head oil)	Winter sperm oil, freed from spermaceti (head oil)	Winter sperm oil, treated with Na_2CO_3 (head oil)	Winter sperm oil, treated with Na_2CO_3 (intestine oil)
Sp. gr.	——	$d_4^{15} = 0.887$	$d_4^{15} = 0.856$	$d_4^{15} = 0.917$
Refr. index	——	$n_D^{26} = 1.459$	$n_D^{26} = 1.460$	$n_D^{28} = 1.470$
Acid value	0	0.5	0.2	0.2
Sap. value	135 3	152.8	153.4	148.3
Iod. value	17.0	76.9	66.2	90.6

iii) Finback whale oils purified with Na_2CO_3: The analysis of the samples treated with Na_2CO_3 in the above method (ii):

	Purified finback whale oils	
	The blubber oil	The intestine oil
Sp. gr.	$d_4^{15} = 0.919$	$d_4^{15} = 0.918$
Refr. index	$n_D^{28} = 1.474$	$n_D^{28} = 1.474$
Acid value	0.2	0.2
Sap. value	184.3	182.5
Iod. value	93.0	102.5

The results indicated that the glycerides are not destroyed.

Feeding experiments: Feeding experiments were carried out in the similar manner as described above. The diets consisted of:

Potato starch (J. P. IV.)	70 g	65 g
Fish meat protein (bonito meat)	15 ″	15 ″
McCollum's salt mixture	4 ″	4 ″
Yeast as d scribed above	2 ″	——
Oryzanin solution	——	5 cc
Waxes prepared as described above	10 g	15 g
Biosterin dissolved in olive oil per os daily	1 mg	1 mg

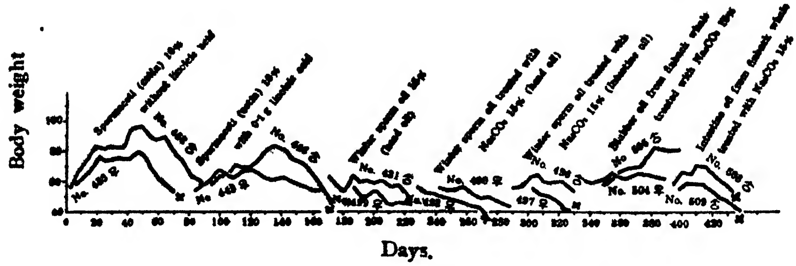


Fig. 9. The growth curves of the rats fed on diets containing purified whale oils.

Besides, a little linoleic acid was added to each diet, because it was proved to be an indispensable food factor, the absence of which causes a disease like avitaminosis.

From the above experiments it can be seen that the pure waxes have no noticeable nutritive value upon rats, and seborrhoea seems sometimes to be produced by other fats or oils which contain waxes either in small amount or free from it (Fig. 9∼14).

(III) *Experiments with fatty alcohols.*

Pure waxes had neither any pronounced nutritive value upon rats, nor seemed to produce seborrhoea. So, the authors carried out some experiments with purified fatty alcohols prepared from the head oil (sperm whale) by saponifiction. As it was shown by M. Tsujimoto and Y. Toyama, the principal components of the head oil from sperm whale are cetyl, octadecyl and oleic alcohols, so the authors prepared these alcohols in pure state and separately tested on rats.

Cetyl alcohol: The solid waxes isolated from sperm oil was saponified with alc. KOH in the usual way, and the fatty acids were precipitated as Ca-soaps with hot alcoholic solution of $CaCl_2$; filtered and the filtrate, after the removal of ethyl alcohol, was extracted with ether, and the etherial extract after washing with water was evaporated to dryness. The residue consisted chiefly of cetyl and octadecyl alcohols. It was recrystallized from acetone, and converted into the corresponding acetate by boiling with three times its amount of acetic anhydride for three hours. The acetylated products thus obtained were fractionally distilled in vacuum. The fraction boiling at

166～7°C (2 mm) having the iodine value 7.4～10 was saponified and subjected to distillation and the distillate was used for animal experiments; b p (3 mm)=161～2°C. In order to determine the purity of this preparation, it was again converted into acetate and the iodine value was determined with the following results.

0.1215 g Subs. gave 1.1 c.c. $Na_2S_2O_3$ (1 c.c.=12.58 mg iodine) iodine value=11.4
0.1206 " " " 1.1 " " " " =11.4

Oleic alcohol: Oleic alcohol was prepared in the similar manner as that of cetyl alcohol from the aceton-soluble portion of the nonsaponifiable matter, separated from the liquid waxes in sperm oils. After repeated distillation of the acetylated product, the preparation gave the following constants: b p (4 mm)=180～5°C, d_4^{15}=0.894, n_D^{20}=1.4532, iodine value=69.5～79.5 (cal. 81.9). The preparation used in the animal experiment was also prepared from the above one by saponifying and redistilling: b p (4 mm)=178～180°C. The purity of the preparation was confirmed by converting it into the acetate: b p (3 mm)=180～182°C.

0.1148 g Subs. gave 6.5 c.c. $Na_2S_2O_3$ (1 c.c.=12.58 mg iodine) iodine value=71.2
0.1185 " " " 6.7 " " " " =71.1

Oleic alcohol (synthetic): Synthetic oleic alcohol was prepared from ethyl oleate by reduction with Na in dry ethyl alcohol. After the removal of alcohol the sample thus obtained was washed with alkali, dried over anhydrous Na_2SO_4, and distilled in vacuo: b p (4 mm)=177～8°C. Then, the preparation was analyzed as the acetate: b p (3 mm)=180～2°C, d_4^{15}=0.889, n_D^{20}= 1.4543.

0.1139 g Subs. gave 7.8 c.c. $Na_2S_2O_3$ (1 c.c.=12.58 mg iodine) iodine value=86.1
0.1133 " " " 7.8 " " " " =86.6
(cal. 81.9)

Feeding experiments: The feeding experiment of rats with the fat-deficient diet supplemented with 5% and 10% fatty alcohols were as follows: The diets consisted of:-

Potato starch (J. P. IV.)	70 g	75 g	80 g
Bonito meat protein	15 g	15 g	15 g
McCollum's salt mixture	4 "	4 "	4 "
Yeast as above described	2 "	2 "	2 "
Fatty alcohol (cetyl or oleic alcohol)	**10 g**	**5 g**	**0 g**
Biosterin dissolved in olive oil per os daily	1 mg	1 mg	1 mg

Preparation used in the expt.		Body wt. at the beginn. of the expt. (av. of 4 rats)	Body wt. at the end of the expt. (av. of 4 rats)	Days of living (av. of 4 rats)
Cetyl alcohol	10%	59 g	44.0 g	11.0
(the head oil f. sperm whale)	5%	58.5 g	45.5 g	10.0

Oleic alcohol 10%	58 g	44.0 g	3.5
(〃) 5%	59 g	45.7 g	3.7
Oleic alcohol (synthetic) 10%	58 g	40.0 g	5.0
Control 0%	61 g	105.0 g	50.0

Moreover, in order to determine the noxious effect of oleic alcohol on rats, when fed on a diet containing butter, rats were fed on a basal diet consisting of starch 75 g, bonito meat protein 15 g, butter 5 g, McCollum's salt mixture 4 g, and commercial oryzanin solution 5 c.c.. The sample oleic alcohol 0.1 g was supplied for each rat per os daily.

The preparation used in the expt.	Initial body wt.	Max. body wt. during the exp.	Body wt. at the end	Duration for expt.
Oleic alcohol	61 g	66 g	63 g	20 (days of living)
(the head oil sperm whale)	57 g	63 g	53 g	21 (〃)
Control	54 g	69 g	69 g	25

From the above result, it was confirmed that free fatty alcohols especially oleic alcohol gave toxic effects upon animals.

(IV) *Injection experiments with oleic alcohol.*

This experiment was conducted with the object of determining the possible role of oleic alcohol in the production of seborrhoea in rats fed on the diet supplied with sperm oils. 2 c.c. oleic alcohol or its acetate prepared from the sperm oil was injected to the peritoneal cavity of rats which were previously fed on an adequate diet. No seborrhoea was produced in any of the rats (4 rats); and three of them died after 10～40 hours and the last one injected with 2 c.c. oleic acetate died after 6 days. So it was also concluded that oleic alcohol itself would not produce seborrhoea in rats.

(V) *Effect of linoleic acid upon seborrhoea of rats supplied with sperm whale oils.*

An additional experiment was repeated to see the effects of linoleic acid and yeast on the sick rats, because they were recently recognized to be an indispensable food factor like vitamins. Rats were fed on the similar diet as above described: potato starch 65 g, fish meat protein 15 g, McCollum's salt mixture 4 g, dry yeast extracted with ether 2 g, sperm whale oil or finback whale oil 15 g, and in addition to the basal diet biosterin 1 mg and

pure linoleic acid* 50～100 mg were supplied to every rat per os daily. The rats given the head oil from sperm whale exhibited seborrhoea after 3 weeks; but on the contrary, the one given the intestine oil from sperm whale and finback whale oils was not observed to show the skin symptom for the entire feeding period of about 100 days. (Fig. 15～16).

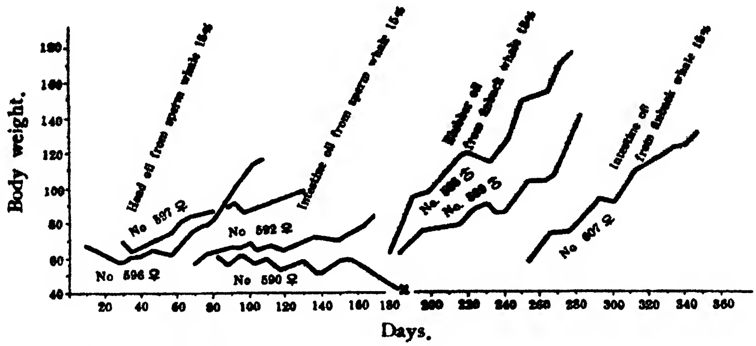


Fig. 15 The growth curves of rats fed on diets containing 15% sperm whale or finback whale oils, supplemented with a little linoleic acid and dry yeast.

Summary

1. The sperm whale oils produced seborrhoea in rats and exhibited the retarding action upon the growth of rats. Finback whale oils (especially the intestine oil), on the contrary, indicated a pronounced nutritive value.

2. In order to determine whether waxes might exhibit any nutritive activity in rats, the head oil from sperm whale was freed from spermaceti (cetin) at a temperature of 0～5°C, and expressed oil was used as the sample of winter sperm oil for investigation. Moreover, the above four kinds of oils were separately treated with Na_2CO_3 in boiling water, and the refined oils so obtained were used for the experiments. Pure waxes prepared from sperm oil when given per os did not show the said symptom, especially when a little linoleic acid and dry yeast were administered with the waxes; so that, seborrhoea developed in rats fed on the diets containing large amount of sperm oils does not seem to be due to the high content of waxy substances in the materials.

3. Fatty alcohols especially unsaturated fatty alcohols like oleic alcohol which is a component of sperm whale waxes, have marked toxic property, but do not produce seborrhoea in rats when given per os or injected. As the waxes as such had no noxious effect, it was concluded that the waxes were not assimilated by the animals; otherwise, the diet containing sperm

* Pure linoleic acid was specially prepared by the present author from soy bean oil by the bro nination method.

Fig. 2—Head oil from sperm whale 15% No. 363. body wt. 49 g (after 9 days)

Fig. 3—Intestine oil from sperm whale 15% No. 390. body wt. 55 g (after 11 days).

Fig. 4—Blubber oil from finback whale 15% No. 353. body wt. 102 g (after 48 days).

Fig. 5—Intestine oil from finback whale 15% No. 355 body wt. 121 g (after 48 days).

Fig. 6—Blubber oil from finback whale 15% No. 351. body wt. 64 g (after 75 days).

Fig. 7—Intestine oil from finback whale 15% No. 355. body wt. 152 g (after 75 days).

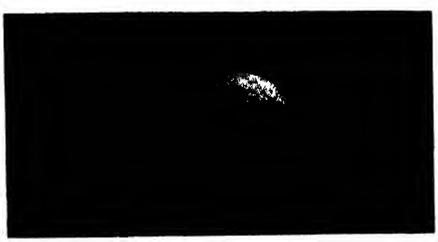


Fig. 10—Spermaceti (cetin) 10% added with yeast 2% No. 452. body wt. 89 g (after 52 days).

Fig. 8—Intestine oil from finback whale 15% No. 355. body wt. 180 g (after 128 days).

Fig. 11—Spermaceti (cetin) 15% Oryzanin 5 cc added No. 428 body wt. 65 g (after 30 days).

Fig. 12—Head oil from sperm whale 15% Oryzanin 5 cc added No. 490 body wt. 51 g (atter 30 days).

Fig. 13—Intestine oil from sperm whale 15% treated with Na_2CO_3 Oryzanin 5 cc added No. 496 body wt. 61 g (after 30 days).

Fig. 14—Blubber oil from finback whale 15% treated with Na_2CO_3 Oryzanin 5 cc added No. 504 body wt. 62 g (after 50 days).

Fig. 16—Head oil from sperm whale 15% added with linoleic acid (2 drops) and yeast No. 595 body wt. 53 g (after 3 weeks).

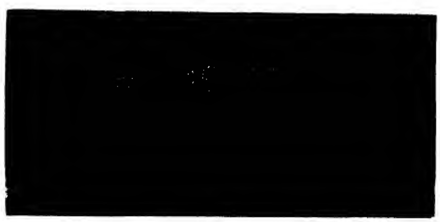


Fig. 17—Intestine oil from sperm whale 15% added with linoleic acid (2 drops) and yeast No. 592 body wt. 69 g (after 5 weeks).

whale oils in sufficient quantity should give more noxious effect.

Moreover, the author has observed that diets supplied with fat consisting of ordinary glycerides have frequently produced the similar symptom in the skin of rats; and on the other hand, that the symptom was prevented by the addition of a small amount of yeast and linoleic acid even when given sperm oils.

The author expresses his sincere thanks to Prof. U. Suzuki for his kind advice and encouragement throughout the work. The author is also indebted to Mr. K. Tago and to the Tōyō Hogei Kaisha for the kind supply of reliable whale oils in fresh condition.

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(1) J. Soc. Chem. Ind. Japan, Suppl. 24, 11~15, (1921).
(2) J. Soc. Chem. Ind. Japan, Suppl. 28, 13~16, (1925); 30, 137~8, (1927).

On the Ergosterin Content of Various Edible Mushrooms in Japan.

By Midzuho SUMI.

(Received February 17, 1933.)

Some years ago[1], the author had isolated ergosterin from *Cortinellus Shiitake*, carefully studied its chemical nature, converted it into vitamin D by irradiation with ultra-violet rays, and proved it to be identical with the preparations obtained from ergot or from yeast by other authors. Later, it was observed that this substance is widely distributed in many other mushrooms, either cultivated or grown wild in various districts of Japan. As these mushrooms are not only consumed to a large extent as a favourite food by our people but also some of them are exported to other countries in a notable amount, it seems desirable, from the stand point of nutrition and public health, to determine their ergosterin content quantitatively. As there is not yet reliable method known for this purpose the author has adopted the digitonin method which is commonly used for the determination of sterins.

Since there is no noticeable amount of sterin other than ergosterin, detected in these fungi, the author presumes that the result obtained by the above method will suffice to represent the approximate amount of ergosterin.

The method of estimation is as follows:

1) The dried and finely powdered material (50 g for each determination) is thoroughly extracted with ether in Soxhlet apparatus, the etherial extract is then evaporated and the residue thus obtained is dissolved in 95% alcohol and filled up to a definite volume.

2) A portion of this solution serves for the determination of free ergosterin. For this purpose, it is treated with an excess of 1% digitonin solution in 90% alcohol, the free ergosterin being thus completely thrown down as the digitonid. After standing overnight in a cool place, the precipitate is collected on a weighed filterpaper, washed with alcohol and ether and dried at 100° to constant weight. From the weight of the digitonid, the percentage of free ergosterin in the original sample is calculated.

3) For the determination of total ergosterin, a part of the latter which exists in the ester form must be previously saponified; otherwise, it is not precipitated by digitonin. For this purpose the above alcoholic solution is boiled with 20% caustic potash for one hour, and after cooling, it is diluted with water and thoroughly extracted with ether. The ergosterin together with other unsaponifiable matters is thus taken up in ether. The etherial extract thus obtained is, after dehydration, evaporated and the residue is again dissolved in 95% alcohol and treated with the digitonin solution as stated above. The difference between the total and the free ergosterin represents the quantity of ergosterin in ester form. For yeasts and spores of *Aspergillus oryzae*, the above method cannot be directly applied, so it is modified as follows;

The dried material (50 g) is suspended in 200 c.c. of 95% alcohol and boiled for 2 hours with concentrated caustic potash (40 g). The supernatant solution is decanted and then the insoluble residue is again boiled with caustic potash likewise. The combined extract thus obtained is then evaporated at low temperature, diluted with a large quantity of water and thoroughly extracted with ether. The etherial solution is dehydrated, evaporated and the residue is dissolved in 95% alcohol, filled up to a definite volume, and treated with the digitonin solution as stated above. In this way, the amount of total ergosterin is obtained.

I. Ergosterin Content in different Parts of Cortinellus Shiitake.

Full grown "Shiitake" was divided into three parts, i. e.,

a) Flesh together with peel. b) Gills. c) Stem.

Each part was separately dried, powdered and analyzed with the following results.

Table. I.

Part of body	Relative wt. of each part of fungus	Ergosterin % in		
		Free state	Ester form	Total
a	37%	0.2261	0.9233	0.2494
b	43	0.3013	0.0312	0.3325
c	20	0.1324	0.0136	0.1460

We see from the above result that the ergosterin content is highest in gill while lowest in stem.

II. Ergosterin Content of *Cortinellus Shiitake* at Different Stages of Growth.

The samples for analysis were gathered at four different stages, i. e.,

a) Button stage :– Cap was globular cylindrical form, the diameter of which was 5～6 mm.

b) Young stage :– Cap expands and veil separates from the margin, the diameter 1～2 cm.

c) Mature stage :– Cap is broadly expanded or nearly flat, the diameter of which was *ca.* 4 cm. In this stage the fungus is usually harvested.

d) Old stage :– Over–riped. Cap becoming funnel shaped; the diameter was more than 5 cm.

The analysis of these samples gave the following results.

Table II.

Stage of growth	Ergosterin % in		
	Free state	Ester form	Total
a	0 1572	0.0214	0 1786
b	0 1882	0 0238	0 2120
c	0 2353	0.0286	0 2639
d	0 2525	0.0325	0 2850

The above result shows that the ergosterin content increases with the development of the fungus.

III. Ergosterin Content of Various Mushrooms.

Most of the samples used in this experiment was kindly supplied by Dr. S. Mimura, to whom the author expresses his hearty thanks. For analysis the dried and powdered material was treated as stated above. The results were as follows :–

Table III.

Name	Fresh sample %		Dry Matter Ergosterin %
	Moisture	Ergosterin	
Armillaria Matsudake Ito et Imai	35.32	0.0309	0.2103
Lactarius Hatsudake Tanaka	91.56	0.0162	0.1915
Tricholoma Shimeji Kawamura	93.42	0.0147	0.2237
Armillaria mellea (vahl) Quelet	87.31	0.0158	0.1243
Boletus edulis (Bull) Fr.	18.72	0.1017	0.1252
Psalliota campestris (Linn) Fr.	91.85	0.0142	0.1751
Rhizopogon rubescens Tul.	73.20	0.0424	0 1582
Tricholoma colossum Fr.	88.23	0.0204	0.1734
Cortinellus Shiitake P. Henn (*Cortinellus Berkeleyanus* Ito et Imai)	70 87	0 0769	0.2639
Polyporus frondosus (Fl. Dan.) Fr.	89.50	0.0106	0 1008
Pholiota discolor peck. Young	91.50	0.0164	0.1932
Pholiota discolor peck. Mature	90.30	0.0228	0.2352
Polyporus varius (pers) Fr.	16 55	0.0778	0 0932
Pleurotus ostreatus (Jacq) Sacc.	10 53	0.1277	0 1428
Hydnum aspratum Berk.	13.10	0 2331	0 2683
* *Polystictus versicolor var nigricans* Losch	17 46	0 0423	0.0513
(2) Spores of *Aspergillus oryzae*	17.82	0.3304	0 4021
** Dried Baker's yeast	7 87	0.3369	0 3657

Conclusion.

The ergosterin content of *Cortinellus Shiitake* varies for different parts of body and it increases a little as the latter grows.

By comparing the ergosterin content of various kinds of edible mushrooms, it was found that they range between 0.1 to 0.4% of the dry materials.

The author wishes to express his sincere thanks to Professor U. Suzuki for his kind guidance throughout this work, and also to Dr. S. Mimura for many valuable advices and conveniences.

Reference

(1) Biochem. Z., **204**, 397, (1929); Proc. Imp Acad., **4**, No. 3, (1928).
(2) Biochem. Z., **195**, 161, (1928).

* *Polystictus versicolor var nigricans* does not belong to the edible mushroom; it grew on the decayed oak stump after Shiitake was harvested.

** As the spores of *Aspergillus oryzae* and yeast contain besides ergosterin many other sterins, the figures given here represent only approximate values.

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Studies on Experimental Rickets. IV

On the Occurrence of Ergosterol in Shoyu Oil.

By

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(Received April 4, 1933.)

Introduction.

Shoyu oil is an oily substance which rises to the top of shoyu or soy sauce when "moromi"* is filtered by means of a hydraulic press. The oil is therefore obtainable as a by-product of soy sauce brewing, and its annual production may be estimated at about 100,000 koku or 16,000 tons in Japan proper alone. It is a dark brown oil with a pungent soy sauce-like smell, and its boiling point is considerably lower than those vegetable oils, which is due to the fact that a majority of its fatty acids is combined with ethyl alcohol instead of glycerine. The use of shoyu oil is rather limited owing to its unpleasant odour and low boiling point, the oil being at present utilized almost exclusively as a raw material for the manufacture of powdered soap of cheaper grades.

During the course of spectrographical and biological examinations of sterols isolated from different sources, we found that a significant amount of ergosterol was present in the sterol obtained from shoyu oil. We thought therefore that it might be of some economical and scientific significances to determine the value of shoyu oil as a raw material of obtaining vitamin D preparations. Accordingly we tried to estimate the ergosterol content of shoyu oil supplied by several different brewers and then to demonstrate on the animal its profound antirachitic activities conferred by ultra-violet irradiation. We also discussed in the present paper the origin of ergosterol found in shoyu oil, and speculated on its economical value as a source of ergosterol in comparison with various other substances containing ergosterol.

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* For the brewing of soy sauce tane-koji or the culture of aspergillus oryzae is added to a mixture of nearly equal parts of cooked soya bean and roasted wheat, and allowed to stand in a cellar until mixture is slightly covered with the fungus, which is then steeped in a large vat of brine and left to ferment for about a year. After the ripening period the fermented mass which is called "moromi" is transferred into a press and liquid sauce is pressed out.

Experimental Results.

(A) Chemical Examinations.

The specimens of shoyu oil supplied by four different brewers had the general characteristics given in Table I.

Table I.

	I	II	III	IV
Specific gravity (15°C)	0.892	0.899	0.896	0.896
Acid value	64.2	61.6	57.6	48.5
Saponification value	183.1	183.3	184.4	183.2
Reichert-Meissl value	——	0.33	——	——
Iodine value (Wij's)	112.5	117.3	117.600	125.8
Unsaponifiable matter	3.03%	3.87%	2.72%	4.130%

Sterol was isolated from the unsaponifiable matter by means of recrystallizations by using acetone and then alcohol as solvents (yield, about 2% of the oil). It gave an intense colour with the Liebermann-Burchard's reagent and also it possessed the Rosenheim's chloral hydrate and trichloroacetic acid reactions both specific for ergosterol. A specimen of sterol melted at 136~7°C and showed $[\alpha]^{25°}_{5461} = -12.85°$, while its acetate had m.p. of 131~2°C and $[\alpha]^{24°}_{5461} = -13.35°$.

(B) Spectrographical Examinations.

The spectrographical examinations were carried out on the ether solutions of both shoyu oil and its sterol, employing a hydrogen vacuum tube as the source of ultra-violet light. It was observed that both of these materials had maximum absortion bands at 293, 282, 270 and 260 $\mu\mu$; the positions being identical with those of ergosterol.

The estimation of ergosterol content of test materials was based on the concentration as well as on the thickness of absorption layer of their solutions at which the 282 $\mu\mu$ band shows the similar intensity to that given by a standard solution of ergosterol at a certain thickness. Four specimens of shoyu oil above mentioned were observed to contain ergosterol at 1.1, 1.3, 1.4 and 1.1%: and three specimens of sterol separated from different samples contained ergosterol at 19.8, 16.7 and 20.0%.

(C) Biological Examinations.

The antirachitic properties of test materials were demonstrated on rats, following the method previously reported(1). It was observed, as indicated in Table II, that while the non-irradiated shoyu oil had no appreciable degree of antirachitic properties, the same oil after one hour's exposure to the light of a mercury vapour lamp became exceedingly great in the above properties,

the daily doses of 1/10 mg being sufficient to produce a constant healing of experimental rickets in rats. The sterol similarly irradiated with ultra-violet light was found to be more potent in the activities, doses such as 1/100 mg per day being always effective in curing rickets.

Table II.

Test material	Daily dose	Rat No.	Radiographic findings at the end of	
			Rachitic period	Test period
Shoyu oil	10% of diet	BBN02	Moderate rickets	No healing
	〃	BBN10	〃	〃
	5% 〃	BBN12	〃	〃
	〃	BBN21	〃	〃
	100 mg	BBL02	〃	〃
	〃	BBN10	〃	〃
Irradiated shoyu oil	10 mg	BBC00	Slight rickets	Complete healing
	〃	BBC01	Moderate rickets	〃
	〃	BBC12	〃	〃
	〃	BBC21	Slight rickets	〃
	1 mg	BBK00	Moderate rickets	〃
	〃	BBK01	〃	〃
	〃	BBK02	Slight rickets	〃
	〃	BBK10	Moderate rickets	〃
	0.5 mg	BBL00	〃	〃
	〃	BBL01	Marked rickets	〃
	〃	BBN00	Slight rickets	〃
	〃	BBN01	〃	〃
	0.1 mg	BBR00	Moderate rickets	〃
	〃	BBR01	〃	Advanced healing
	〃	BBR02	〃	〃
	〃	BBR10	〃	Complete healing
Irradiated sterol	1 mg	AMF02	Moderate rickets	Complete healing
	〃	AMF10	Marked rickets	Advanced healing
	〃	AMG00	Moderate rickets	Complete healing
	〃	AMG01	Marked rickets	〃
	0.1 mg	AME02	Moderate rickets	〃
	〃	AME10	Marked rickets	〃
	〃	AMF00	〃	〃
	〃	AMF01	Slight rickets	〃
	0.01 mg	AMD02	Moderate rickets	〃
	〃	AMD10	Marked rickets	Slight healing
	〃	AME00	Moderate rickets	Complete healing
	〃	AME01	〃	〃
	0.001 mg	AMQ00	〃	Advanced healing
	〃	AMQ01	〃	Slight healing
	〃	AMQ02	〃	Advanced healing
	〃	AMQ10	Slight rickets	Complete healing

Discussion

The sterol of shoyu oil was first examined by Tsujimoto and Ueno[2] and then by Fukai[3]. These investigators were all of the opinion that it was nothing but phytosterol. Fukai[4] lately investigated more thoroughly on the sterol obtained from the oil of shoyu press-cake and was able to iso-

late two kinds of sterols. The one metled at 95~97°C and showed $[\alpha]_D=$ +20, and the other had m.p. of 137.5~140°C and $[\alpha]_D^{19°}=-44$. Based on these facts as well as on the results of elementary analyses of the sterols he conjectured that coprosterol might be present in the former substance and ergosterol in the latter.

The results of biological investigations conducted by us were found to be consistent with our spectrographical findings, which showed that in shoyu oil more than 1% of ergosterol was present. It is rather surprising to find that shoyu oil contains ergosterol in such large quantities, which interested us to speculate on its origin. As above cited, the chief organic raw materials used for the brewing of soy sauce are soya bean and wheat in which oils are contained at about 18 and 2% respectively. We previously proved that ergosterol was present in soya bean oil(5) at about 0.0005~0.001% and in wheat bran oil at about 0.05~0.1%. A mixture of equal parts of soya bean and wheat is therefore capable of supplying oil which contains ergosterol at a level of about 0.0055~0.011%, a great discrepancy in the ergosterol content being thus found between the oil supplied by the mixture and shoyu oil. It is therefore reasonable to suppose that a greater part of ergosterol found in shoyu oil is derived from the sources other than these raw materials.

The micro-organisms which participate in the soy sauce brewing, such as, aspergillus oryzae, various yeasts (chiefly zygosaccharomyces) and bacteria are shown by a number of investigators to contain ergosterol in their bodies. It is also shown by Takahashi and Lin(6) that aspergillus oryzae can synthesize ergosterol from the nutrient media containing no organic substances except sucrose. It is concluded from these facts and considerations that a major part of ergosterol in shoyu oil may be produced by various micro-organisms during the period of fermentation, only a minor part being derived from the raw materials.

The following substances may be regarded as important raw materials which can be utilized for the commercial production of vitamin D preparations in Japan: beer yeast, ergot, tane-koji or the culture of aspergillus oryzae, cotinellus shiitake, armillaria edodes, saké press-cake, wheat bran and rice polishings. The Dairen market price and ergosterol content of these materials as well as the cost of materials equivalent to 1 kg of ergosterol were compared with those of shoyu oil. These comparisons are shown in Table III, from which it must be admitted that shoyu oil is capable of supplying ergosterol much cheaper than any of the other substances above mentioned. We therefore think that shoyu oil will be highly esteemed in the near future as one of the most valuable sources of obtaining vitamin D in the orient.

Table III.

Raw Material	Price of material per kg (yen)	Ergosterol content (%)	Cost of material equivalent to 1 kg of ergosterol (yen)	Investigator
Shoyu oil	0.16	1.0 ~1.5	10.7~16.0	Authors
Beer yeast (dry)	2.67	0.2 ~0.4	668~1,335	Sumi, Authors
Ergot	4.00	0.13	3,077	Hart and Heyl
asp. oryz. (dry)	——	0.25	——	Takahashi and Lin
Spores of asp. oryz.	——	0.08	——	Sumi
Tane-koji (dry)	2,50	0.05 ~0.08	3,125~5,000	Authors
Cortinellus shiitake (dry)	3.00	0.26	1,153	Sumi
Armillaria edodes (dry)	2 70	0.21	1,286	Sumi
Saké press-cake (dry)	0.45	0.01 ~0.02?	2,250~4,500	Takahashi and Lin
Wheat bran	0.03	0.003~0.006	500~1,000	Authors
Rice polishings	0.04	0.002~0.004	1,000~2,000	Authors

Summary.

(1) It was spectrographically estimated that several specimens of shoyu oil supplied by different soy sauce brewers contained ergosterol at a level of about 1.1~1.4% and those of sterol isolated from the oil at about 16.7~20.0%.

(2) Both shoyu oil and its sterol were shown to be rendered highly antirachitic by means of ultra-violet irradiation, daily doses of 1/10 mg of irradiated oil and 1/100 mg of irradiated sterol being sufficient to produce a constant healing of rickets in rats.

(3) There are several evidences to indicate that a major part of ergosterol found in shoyu oil can be produced by aspergillus oryzae and other micro-organisms during the fermentation and only its minor part to be derived from the raw materials of soy sauce.

(4) It is suggested that shoyu oil can be utilized as the cheapest source of ergosterol in Japan among the known substances containing ergosterol.

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Studies on Blood Pressure Decreasing Substances.

Part I. The presence of blood pressure decreasing substances in yeast cell.

By

Masao TOKI, Tohkichi MIYOSHI and Nagao UYEDA.

(Received April 17, 1933.)

When dried yeast (Sac. cereviciae) is administrated orally per kg ca. 0.05 g to a rabbit the blood pressure is more or less decreased.

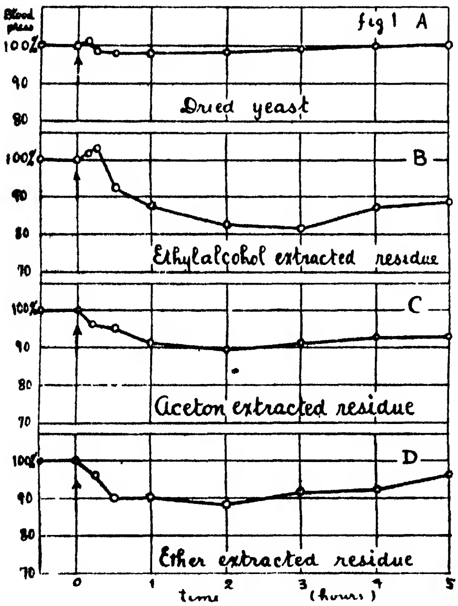


Fig. 1 Effects of dried yeast and its organic solvent insoluble part on the blood pressure of rabbit. per kg 0.05 g per os.

This blood pressure decreasing activity is pronounced in some degree by extracting with organic solvents such as alcohol, acetone, ether etc. as shown in fig. I.

It is proved also true in the case of ten different species of yeast as shown in fig. 2.

The rise and fall of blood pressure afecting substances during the course of autolysis of fresh yeast is shown in fig. 3. Thus ; pure culture of Sac, cereviciae is subjected to autolysis in a thermostat regurated at 40°C., eight samples are taken in the course of a handred hours, and are dried in a vacuum desiccator under special care. Every sample is divided in two parts, one as it is (broken lines), another is extracted with alcohol to remove the soluble matters (full lines). It is noticeable that the effects of autolysates of different stages are very complicated while corresponding alcohol insoluble parts decrease the blood pressure of rabbit throughout the stages.

It suggests intermediate formation of blood pressure increasing substance, with unstable nature, during the autolysis of yeast.

Since it is impossible to avoid a certain degree of autolysis in preparing dried yeast in bulk, the obscurity of the effects of dried yeast on blood pressure become complehensive.

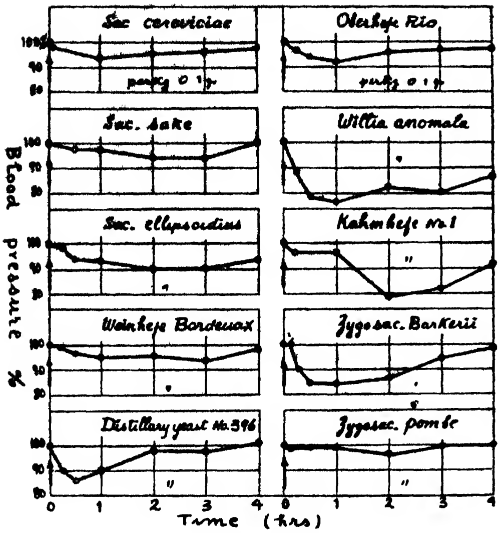
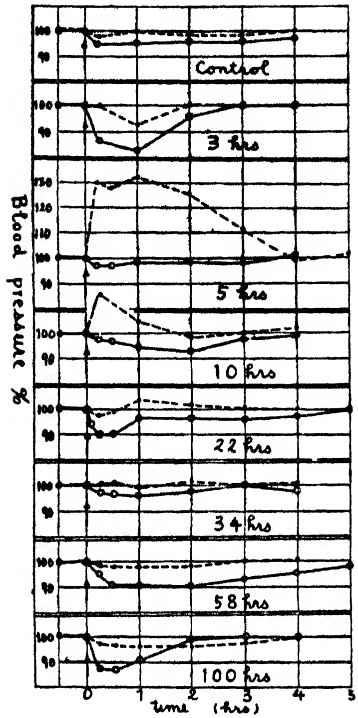


Fig. 2 Effects of alcohol insoluble part of dried yeasts of different species on the blood pressure of rabbit. per kg 0 1 g per os



---------- Autolysate.
———— Alcohol insoluble part of autolysate.

Fig. 3 Blood pressure affecting substances in the course of autolysis of fresh yeast. Rabbit; per kg 0.1 g per os

Studies on Blood Pressure Decreasing Substances.

Part. 2. Yeast nucleic acid.

By

Masao Toki and Tohkichi Miyoshi

(Received April 17, 1933.)

Sodium nucleate.

When an aqueous solution of sodium nucleate is administrated to a rabbit

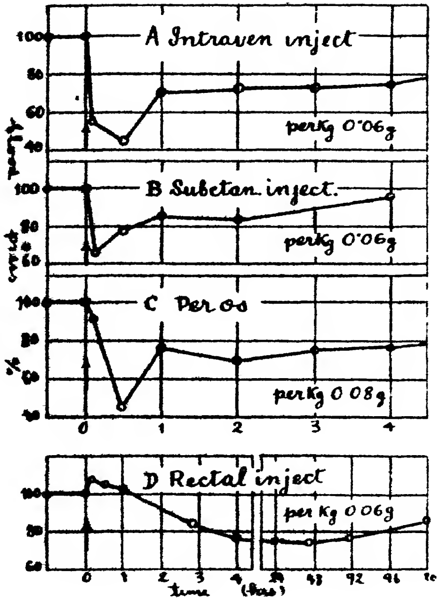


Fig. 1 Effects of Na-n[illegible]ate on the blood pressure of rabbit.

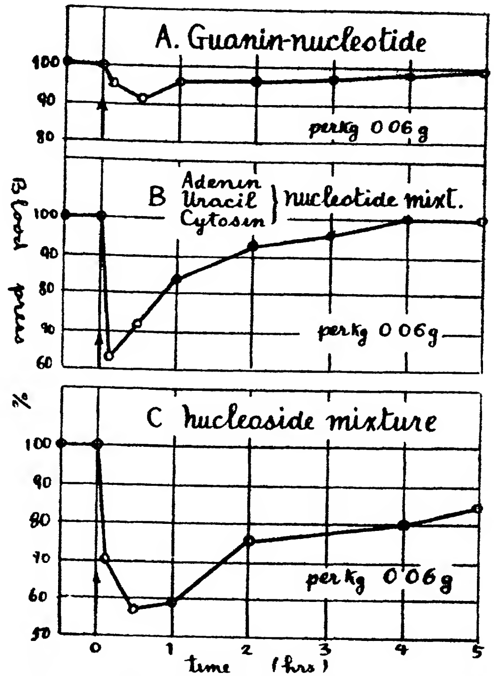


Fig. 2 Effects of intermediate hydrolytic products of nucleic acid on the blood pressure of rabbit. Intraven. inject.

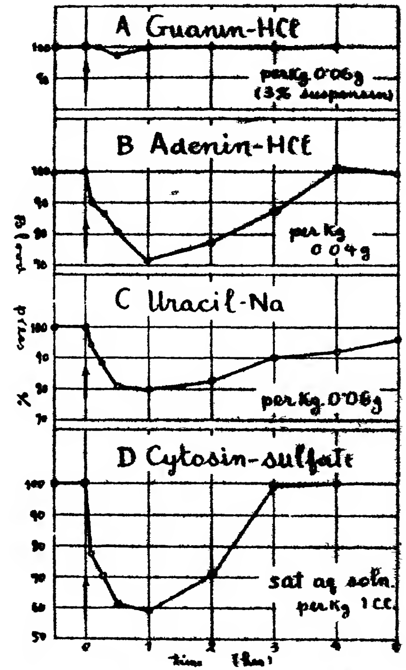


Fig. 2 Effects of intermediate hydrolytic products of nucleic acid on the blood pressure of rabbit. Intraven. inject.

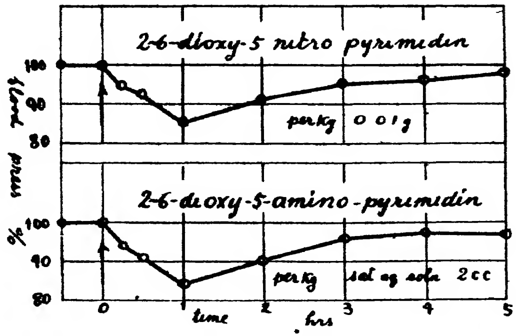


Fig. 4 Effects of synthetic pyrimidins on the blood pressure of rabbit. Intraven inject.

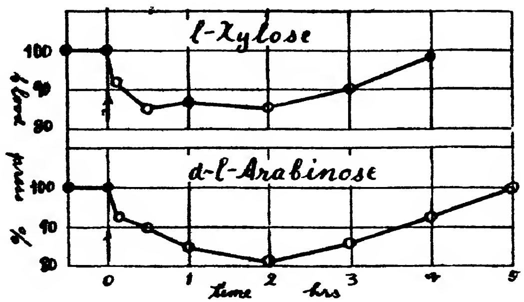


Fig. 5 Effects of aldopentoses on the blood pressure of rabbit. per kg 0.2 g ; intraven. inject.

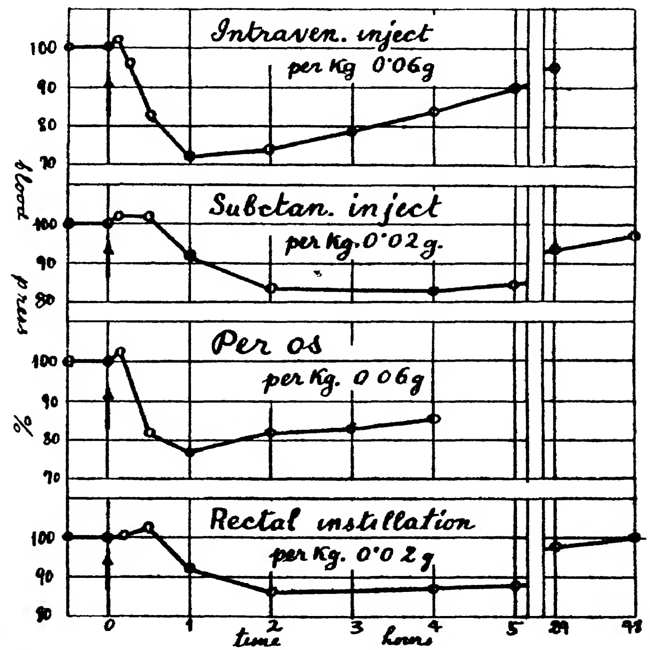


Fig. 6 Effects of Na-phosphate on the blood pressure of rabbit.

by one of the means of intraven. inject., subctan. inject, per os or rectal instillation, the blood pressure is remarkablly depressed in every case (fig. 1).

Hydrolytic products of nucleic acid.

(1) Intermediate hydrolytic products.

Both nucleotides and nucleosides decrease the blood pressure of rabbit when injected intravenously (fig. 2).

(2) Final hydrolytic products.

a) Bases.

Adenin, uracil and cytosin have strong blood pressure decreasing activity while that of guanin is obscure (fig. 3).

According to the fact that two of the synthetic pyrimidins, 2,6-dioxy-5-nitro-pyrimidin and 2,6-dioxy-amino-pyrimidin, also decrease the blood pressure, it is likely to suppose that the blood pressure decreasing activity is an attribute of pyrimidins (fig. 4).

b) Pentose.

Unexpectedly, when an aqueous solution of l-xylose is injected intravenously the blood pressure of the rabbit is decreased and same result is observed in the case of d. l-arabinose (fig. 5). The similarities of intensity of the effect and the shape of the curves suggest aldopentoses, including d-ribose, generally have blood pressure decreasing activity.

c) Phosphoric acid.

When an aqueous solution of sodium phosphate is administrated to a rabbit by one of the means of intraven. inject., subctan. inject., per os or rectal instillation a remarkable depression of blood pressure is observed in every case (fig. 6).

Regarding to the biochemical importance an independent presice report concerning only to the phosphoric compounds will be contributed before long.

Sterilising Action of Acids. III Report.

Sterilising action of saturated monobasic fatty acids on putrifactive bacteria, Bac. typhosus and V. cholerae.
(The Second Report.)

By

Sogo TETSUMOTO

(Received April 19, 1933.)

I reported in the preceding edition[1] concerning the sterilising action of

saturated monobasic fatty acids (from formic to capric) on putrifactive bacteria, Bac. typhosus and Vib. cholerae. Now I studied the effect of monobasic higher fatty acids, pH, anions and undissociated molecules conserning the sterilising action on microorganisms.

(1) Reagents, rational formulae and pH.

These are noted in next table.

Table 1.

Number of C atom	Acids	Rational formulae	Molecular Weight	pH of saturated solution
C_{11}	Undecylic	$CH_3(CH_2)_9 \cdot CO_2H$	186.231	6.0
C_{12}	Lauric	$CH_3(CH_2)_{10} \cdot CO_2H$	200.250	6.2
C_{14}	Myristic	$CH_3(CH_2)_{12} \cdot CO_2H$	228.294	6.2
C_{16}	Palmitic	$CH_3(CH_2)_{14} \cdot CO_2H$	256.336	6.2
C_{18}	Stearic	$CH_3(CH_2)_{16} \cdot CO_2H$	284.378	6.2

Each acids are soluble extraordinally small quantity in water. Accordingly I made saturated aqueous solutions at 20°C.……saturated solution of undecylic acid and lauric acid $\doteqdot N/100{,}000$. myristic, palmitic and stearic acid $< N/100{,}000$. pH of each acid is determined by Itano's electric quinhydrone method. I could not get acids % C_{13}, C_{15} and C_{17}, so I could not study these acids.

(2) Species of microorganisms and Performance of the experiment.

About these, I took the same process as I reported in the preceding edition[(1), (2)].

(3) Sterilising action of saturated monobasic higher fatty acids.

Results are shown in the following table.

Table 2. Sterilising action of saturated aqueous solution of monobasic higher fatty acids. (at 20°C).

Acids	pH	Surviving period																													
		Staph. c. pyogen.								Prot. vulgar., H.								Bac. typhosus								Vib. cholerae					
		2	3	4	8	9	11	12	d 13	18	h 24	d 2	3	4	5	7	8	24	h 36	d 3	5	6	8	9	10	5	10	m 15	h 2	3	6
Undecylic	6.0	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−
Lauric	6.2	+	+	−	−	−	−	−	−	+	+	±	−	−	−	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−
Myristic	〃	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−	−
Palmitic	〃	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	−
Stearic	〃	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	±
Control		+	+	+	+	−	−	−	−	+	+	+	+	+	±	−	−	+	+	+	+	±	−	−	−	+	+	+	+	+	+

+ …… alive − perished ± sometimes alive and sometimes perished

m …… minute h …… hour d …… day

Namely concerning to the sterilising action, undecylic acid is stronger than any acid from C_{12} to C_{18} on saturated aqueous solution at 20°C.

Lauric acid (C_{12}) is weaker than undecylic acid. Acids of myristic to stearic have no sterilising action. And then acids of palmitic (C_{16}) and stearic (C_{18}) give good condition to alive to microorganisms comparing to the control.

(4) Sterilising action at the same pH of saturated monobasic higher fatty acids.

To see the relation between the sterilising action of each acid and pH, I made aqueous solution of pH 6,2 with each acid.

The results are as shown in table 3.

Table 3. Sterilising action at the same pH of saturated monobasic higher fatty acids.

pH 6.2	Surviving period																							
	Staph. c. pyogen.						Prot. vulgar. H						Bac. typhosus						Vib. cholerae					
	3	4	8	9	12	d 13	h 36	d 2	4	5	7	8	2	3	6	8	9	d 10	10	15	m 90	h 2	3	6
Undecylic acid	±	−	−	−	−	−	+	−	−	−	−	−	±	−	−	−	−	−	±	−	−	−	−	−
Lauric 〃	+	−	−	−	−	−	+	±	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−
Myristic 〃	+	+	+	−	−	−	+	+	+	+	−	−	+	+	+	−	−	−	+	+	+	+	−	−
Palmitic 〃	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Stearic 〃	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−
Control	+	+	+	−	−	−	+	+	+	±	−	−	+	+	±	−	−	−	+	+	+	+	+	+

To make the solution of pH 6.2 with undecylic acid (pH 6,0), water of 2/3 volume must be added to the saturated undecylic acid solution.

Accordingly the molecular concentration of undecylic acid solution becomes diluent. Owing to this fact, the sterilising power of undecylic acid solution on each microoganisms becomes weaker.

Notwithstanding, even if on the same pH, the sterilising power of undecylic acid (C_{11}) is the strongest of all. Next to this, is lauric acid (C_{12}). Acids of palmitic (C_{16}) to stearic (C_{18}) have no sterilising power. But on Vib. cholerae each acids have weak sterilising power.

By these facts we know that the sterilising action of saturated monobasic higher fatty acids concern little about their pH.

(6) Sterilising action of anions of saturated higher fatty acids.

To ascertain the sterilising action of anions of monobasic higher fatty acids, I made saturated solution of Na, K, Ca and NH_4 salts having the same anions of acids. Results of Na salts and K salts are nearly the same, so I denote here the results of Na salt only. Results are as shown in Table 4.

Table 4. Sterilising action of salts. (I) Na salts.

Na-salts	Surviving period: Staph. c. pyogen.							Prot. vulgar. H						Bac. typhosus							Vib. cholerae				
	3	4	5	8	9	13	d 14	2	3	4	5	7	d 8	2	3	6	7	8	9	d 10	10	20	m 30	h 3	6
undecylate	+	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−
laurate	+	+	−	−	−	−	−	+	±	−	−	−	−	+	+	−	−	−	−	−	+	+	−	−	−
myristate	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	−	−	+	+	+	+	−
palmitate	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−
stearate	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−
Control	+	+	+	+	−	−	−	+	+	+	±	−	−	+	+	±	−	−	−	−	+	+	+	+	+

(II) Ca salts.

Ca salts	Surviving period: Staph. c pyogen							Prot. vulgar. H								Bac. typhosus							Vib. cholerae					
	3	4	5	8	9	11	d 12	h 36	d 2	3	4	5	6	7	8	2	3	5	6	8	9	d 10	10	20	m 30	h 2	3	6
undecylate	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	±	−	−	−	−	−
laurate	+	+	−	−	−	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	+	+	−	−	−	−
myristate	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	+	−
palmitate	+	+	+	+	+	±	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	−
stearate	+	+	+	+	+	±	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	−
Control	+	+	+	+	−	−	−	+	+	+	+	±	−	−	−	+	+	+	±	−	−	−	+	+	+	+	+	+

(III) NH_4 salts.

NH_4 salts	Surviving period: Staph. c. pyogen.							Prot. vulgar. H							Bac, typhosus							Vib. cholerae						
	3	4	5	8	9	13	d 14	2	3	4	5	7	8	d 9	2	3	4	6	7	10	d 11	15	20	30	m 45	h 3	6	9
undecylate	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−
laurate	+	+	−	−	−	−	−	+	+	−	−	−	−	−	+	+	−	−	−	−	−	+	+	±	−	−	−	−
myristate	+	+	+	+	+	−	−	+	+	+	+	+	−	−	+	+	+	+	+	−	−	+	+	+	+	+	−	−
palmitate	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−
stearate	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−
Control	+	+	+	+	−	−	−	+	+	+	±	−	−	−	+	+	+	±	−	−	−	+	+	+	+	+	+	+

From the results of Table 4 we find the following facts.

Anions of saturated monobasic higher fatty acids have weak sterilising action from caprylic (C_8) to lauric (C_{12}). But anions of myristic (C_{14}) to stearic (C_{18}) have no sterilising action respectively. But on Vib, cholerae anions of

each acids have weak sterilising action.

The results of Na salts, and NH_4 salts are nearly the same. But if we compare in detail, we know that on NH_4 salts microorganisms survive longest and on Ca salts they perish shortest.

(7) Sterilising action of undissociated melecules.

If we compare the results shown in Table 1 and Table 3, we know that the stearilising power of undecylic acid (C_{11}) and lauric acid (C_{12}) is stronger than their salts respectively. And that their pH has almost no sterilising power. By above facts it is found that the sterilising action of undecylic acid and lauric acid is due to the undissociated molecule besides their anions.

Undissociated molecules of myristic (C_{14}) to Stearic (C_{18}) have no sterilising power. But on Vib. cholerae all these acids have weak sterilising power.

I express my profound thanks to Dr. Y. Tohyama and Dr. S. Kojima for their kind advice on this experiment.

Summaries.

I studied the sterilising action of saturated monobasic higher fatty acids from undecylic (C_{11}) to stearic (C_{18}) on putrifactive bacteria, Bac. typhosus and Vib. cholerae.

These acids are soluble extraordinally small quantity to water So that I studied on their saturated aqueous solution at 20°C.

Results are as follows

(1) On the saturated watery solution of acids at 20°C, undecylic (C_{11}) to stearic (C_{18}), the sterilising action of undecylic acid is the strongest, lauric acid is the next. Acids of myristic (C_{14}) to stearic (C_{18}) have no sterilising power. These acids give rather good conditions to the microorganisms to alive comparing to control.

But for Vib. cholerae, all these acids show weak sterilising action.

(2) On the sterilising action of these acids, pH concerns little.

(3) Anions of acids C_{11} and C_{12} have weak sterilising power. But anions of acids C_{14}, C_{16} and C_{18}, have no sterilising power.

(4) Among salts of Na, K, Ca and NH_4, NH_4 salts are weakest of all coneerning to the sterilisation.

(5) On the sterilising action of undecylic acid and lauric acid, undissociated molecules have also remarkable effect.

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Studies on the Germination of Seeds. Part III.

Organic Bases of the Seeds, and Seedlings of Soy-Bean Germinated in a Dark Room.

By

Shûiku SASAKI

(From the Biochemical Laboratory, Department of Agriculture, Kyûshû Imperial University.)

(Received April 21, 1933.)

Several organic bases contained in the seeds and seedlings of the soy-bean were already detected by E. Schulze[1] and K. Yoshimura[2]. Their investigation were not, however, exhaustive, and the results seemed different on many points. In view of this, I deemed it not only desirable, but also high interesting to make further research on this subject and to determine definitely, if possible, what variation of bases in the seed may occur during germination, and has done so. The results achieved are briefly reviewed in this paper.

A. Seeds.

(1) Specimen.

The kind of specimen used was the *white autumn seeds* produced in Korea. The results of the general analysis are shown as follows.

Water	11.2 %
Crude fat	16.6
Total nitrogen	7.03
Allbuminous nitrogen	6.48
Non-albuminous nitrogen	0.55
A. Nitrogen precipitated by lead acetate	0.16
B. Nitrogen precipitated by phoschotungstic acid after removing the nitrogen A.	0.24
C. Remaining nitrogen	0.15

(2) Seperation of Bases from Other Substances in the Seeds.

5 kg of the seeds were ground, fat and allied substances were extracted off by ether, and the residue was ground again. The fine powder was extracted five times with 15 L of slightly acetated hot water. The extract was clarified with neutral and basic lead acetate and evaporated to 4 L under diminished pressure, and the bases were precipitated by phosphotungstic and sulphuric acids. The Precipitate was decomposed by baryta, and the excess of baryta was removed by CO_2 gas.

(3) **Precipitate by Silver Nitrate (Purine Bases).**

The solution of bases was neutralized with nitric acid and precipitated by silver nitrate. The precipitate was treated with hydrochloric acid, and then subjected to the usual method. Two kinds of hydrochloride were resulted, namely, adenine and guanine hydrochloride, and these were separated by means of fractional crystallization. The adenine and the guanine were identified as their chloraurates, picrates, and by the colour reactions.

(4) Precipitate by Silver Nitrate and Baryta (Arginine Fraction).

The filtrate from the precipitate by silver nitrate was treated with more silver nitrate and an excessive quantity of baryta. The precipitate was decomposed by sulphuric and hydrochloric acids, and the free bases were obtained by the usual method.

(a) Histidine :- The solution of bases was saturated with CO_2 gas, and mercuric chloride was added to it. The precipitate was decomposed by H_2S. After removing mercuric sulfide and clarifying it with tannic acid, crystals of histidine dichloride were obtained by the usual method. This chloride was verified by its chlorine content and colour reactions, and it was identified, also, as its picrate, picrolonate, and mono-chloride.

(b) Arginine :- From the filtrate of the precipitate by mercuric chloride, mercury was removed as sulphide, and arginine was obtained as frabianate. It was identified as its nitrate and copper nitrate.

(5) Filtrate of the Precipitate by Silver Nitrate and Baryta (Lysine Fraction).

From the filtrate of the precipitate of the arginine fraction, silver and barium were removed, and the hydrochloride of bases was obtained by the usual method. It was evaporated to dryness and treated with methyl alcohol to remove a large amount of potassium chloride. The solution of methyl alcohol was evaporated to dryness once more and divided into the following two parts by means of cold anhydrous ethyl alcohol.

(a) Choline :- The soluble part in ethyl alcohol was treated with alcoholic solution of mercuric chloride. The precipitate was filtered and decomposed by H_2S, and hygroscopic crystals of choline chloride were obatined by drying. It was identified as its chloraurate and chlorplatinate.

(b) Trigonelline :- The insoluble part in ethyl alcohol contained trigonelline. It was identified as its normal and basic chloraurate, chlorplatinate, and picrate. In this part, the presence of another base was revealed by chloraurate, but on account of the small quantity of it available, further identification was not performed.

B. Seedlings.

(1) Specimen.

In this experiment two kinds of soy-beans were used, namely, *white autumn*

seeds produced in Korea and *yellow summer seeds* produced in Kagoshima, but the procedures of analysis of the two were exactly same.

Well-selected seeds were sown in a dark room. After 12 days the young plants, having stems and roots of from 12 to 18 cm, were plucked out. There were almost no decayed plants, and the specimens were selected with strict care. The results of the genaral analysis are shown as follows.

Water	87 %
Dry matter	13
In dry matter	
Crude fat	10.5
Total nitrogen	7.53
Albuminous nitrogen	4 26
Non-albuminous nitrogen	3.27
A. Nitrogen precipitated by lead acetate	0.40
B. Nitrogen precipitated by phosphotungstic acid after removing the nitrogen A.	0 96
C. Remaining nitrogen	1 91

(2) Separation of Bases from Other Substances in the Seedlings.

The seedlings, plucked out, selected and washed, were ground at once and extracted with slightly acetated hot water. The following process employed with two cases (3 and 4) were exactly the same as those stated in the preceding paragraphs and are, therefore, not repeated here.

(3) Precipitate by Silver Nitrate (Purine Bases).

(4) Precipitate by Silver Nitrate and Baryta (Arginine Fraction).

(5) Filtrate of the precipitate by Silver Nitrate and Baryta (Lysine Fraction).

The bases of this fraction were, as in the case of seeds, made to the hydrochloride, treated with methyl alcohol, and divided into the following two parts by means of cold anhydrous ethyl alcohol.

(a) The insoluble part in cold anhydrous alcohol (Cadaverine). In this part I isolated a large amount of cadaverine and identified it as its chloraurate, chlorplatinate, and picrate.

(b) The soluble part in cold anhydrous alcohol (Cadaverine and choline). The alcoholic solution was clarified with alcoholic solution of mercuric chloride, and separated into cadaverine and choline by means of chloraurate and chlorplatinate, for the chloraurate of cadaverine is more soluble in water than that of choline and the chlorplatinate of choline is more soluble in water than that of cadaverine. These substances were identified as their several salts.

In this experiment I accertained that Stanék's reagent for choline[3] could not be used successfully for isolating choline from the mixture of choline and cadaverine, because cadaverine is also precipitable with this reagent.

C. Summary and conclusion

(1) Adenine, guanine, histidine, arginine, trigonelline, choline, and a small quantity of an unknown base were isolated from soy-bean seeds; and adenine, guanine, histidine, arginine, choline and cadaverine from its seedlings. I obtained these substances quantitatively as far as possibly could. The results of the experiment are shown in the following table setting them side by side with those of Schulze and Yoshimura.

Table 1.

	Schulze		Yoshimura		Sasaki		
	Seeds	Seedlings	Seeds	Seedlings	seeds	Seedlings	
					A**	A**	B**
Adenine	——	——	0 02	present	0 10	0 55	0 42
Guanine	——	——	——	present	0 06	0 12	0 08
Histidine	——	present	present	——	0 08	1 60	2 01
Arginine	0 33	increase*	0 07	——	1 40	3 33	——
Choline	0 61	1 47	0 08	present	1 33	1 40	1 71
Trigonelline	——	——	0 01	——	0 05	present?	——
Betaine	present ?	——	——	present	——	——	——
Cadaverine	——	——	——	——	——	1 22	1 16
An unknown base	——	present ?	——	——	0 16**	——	——

Remarks -

(1) All figures in the table represent g. of bases isolated from 1 kg of seeds by calculation

(2) Schulze stated only "increase by germination", but did not gave any figures to the amount of arginine in seedlings (* mark).

(3) The figures in the raw under the A** marks above are taken from the result of the experiment with the *white autumn seeds* produced in Korea, and those in the B** mark obtained from that with the *yellow summer seeds* produced in Kagoshima, Japan

(4) The figure 0 16*** are represent the amount of chloraurate of an unknown base.

(2) It will be noted that adenine, guanine, arginine and histidine increase in quantity during germination, and markedly so with histidine.

(3) Choline does not so much increase during germination as Schulze set forth.

(4) A little quantity of trigonelline is found in seeds, but the presence of it in seedlings is questionable.

(5) Cadaverine is contained in large quantities in the seedlings. It has not been detected in any higher plant, so far as known.

(6) Staněk's reagent for choline dose not meet the purpose when cadaverine is present.

(7) An unknown base contained in the seed was isolated, but its veri-

·fication had not been carried out, because the quantity obtained was so small that it was impossible.

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On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon. (Part V).

Detection of Violaxanthin in the yellow Cocoon.

By

Masami OKU

(From the Chemical Laboratory of Gunze Raw Silk Mfg. Co. Ltd., Ayabe-machi, Kyôto-hu, Japan.)

(Received March 27, 1933.)

The natural yellow pigments of the domestic yellow cocoon were hitherto identified by the author as chiefly constituted of xanthophylls, from which crystalline pigment was isolated and identified as lutein (m. p. 193°). The mother liquor of lutein, however, has yet the tinge of deep orange red and deemed me the presence of another more oxygen containing pigment than lutein. I have examined the presence of violaxanthin ($C_{40}H_{56}O_4$) in this fraction and isolated it in minor quantity in the crystalline state, in major in the resinous substance and identified it as violaxanthin, taking advantage of its color reaction against several reagents, comparing with lutein ($C_{40}H_{56}O_2$) and fucoxanthin ($C_{40}H_{56}O_6$).

Experimental.

Procedure of isolation.

To the 85% methanol mother liquor of lutein (of Chinese No. 7 yellow species) was added petroleum benzin (b. p. 70~80°) and as much water as adjusting the concentration of methanol to 70%, rejecting minor quantity of crystals of lutein thereby formed. Then the upper layer of petroleum benzin containing xanthophylls was separated and again agitated with 70% methanol

and again separated the upper. This petroleum benzin solution was washed with water and dehydrated and brought to the chromatographic analysis as Tswett given, taking calcium carbonate as adsorbent. The chromatogram there formed was differentiated into three zones, showing the major part of xanthophyll adsorbed near the top of the adsorbent. This top zone of the chromatogram was taken out and extracted with pure methanol. Methanol solution was again submitted to the fractionation with petroleum benzin and 70% methanol and chromatographic analysis followed as already given. The top zone of the chromatogram was extracted with ether, ether evaporated and the residue was repeatedly recrystallized from pure methanol from which very minute amount of pigment was separated which melts at 185°, leaving the major in the resinous state.

Identification of violaxanthin.

(1) Solvent :- readily soluble in $CHCl_3$, ether, soluble in methanol, ethylalcohol, CS_2, insoluble in petroleum ether.

(2) Absorption spectrum :- three absorption bands were perceived which coincide with those of violaxanthin.

(3) Behavior toward alkali treatment :- showed resistance toward 50% alcoholic potash solution and showed no change or its absorption spectrum which proved to be distinguished from fucoxanthin.

(4) Color reaction :- ether solution of the pigment was treated with following reagents and observed color reaction thereby occurred.

(a) HCl :-

18%	HCl.........	showed no coloration
19%	"	showed indigo blue ring a little later
20.5%	"	" " " " immediately
22%	"	" " " " momentary
25%	"	" " " " strongly & momentary

(b) Formic acid :- to the crystal was added conc. formic acid, when there developed green color first, then dissolved changing to indigo blue coloration.

(c) Picric acid :- ether solution of picric acid was added which resulted olive color after two minutes, yellowish green after ten minutes, which is very stable even later.

(d) Glacial acetic acid :- yellowish first, indigo blue afterward.

(5) Fractional distribution between 70% methanol and equivalent mixture of petroleum ether and ethyl ether :- a part of the pigment was extracted from the later with the former.

On The Natural Pigments of Row Silk Fibre of The Domestic Cocoon (Part VI).

Fading of the Yellow Cocoon.

By

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(Received March 27, 1933.)

Yellow cocoons are faded by the drying process and during their storage. They have, on the other hand, characteristic stimulant odor, so-calld "yellow cocoons' odor". The cause of these facts was searched and verified it as resulting from the oxidation of xanthophyll, which is the main yellow pigment of the yellow cocoons.

Experimental.

(1) Oxidation of xanthophyll.

When lutein ($C_{40}H_{56}O_2$) (m. p. 193°) of the yellow cocoon was exposd to the current of oxygen, its yellow tinge was faded and increased in weight gradually, combining with oxygen atom. After 90 days exposure, it was increased in weight by 35% and a composition of $C_{40}H_{56}O_{15}$, melting near about 90°. This oxidized lutein showed no more absorption spectrum as is characteristic to xanthophyll.

(2) Fading during storage.

Yellow cocoons showed no remarkable fading during storage in carbon dioxide atmosphere even after 150 days, while these showed as much as 13 % fading in oxygen atmosphere after the elapse of the same days.

(3) Fading by drying process.

When fresh yellow cocoons were dried for 4 hours at 70°, they lost the yellow tinge as much as 13%.

(4) Presence of xanthophyll on the way of oxidation.

The natural yellow pigments present in the mother liquor of crystalline lutein showed the average composition of $C_{40}H_{56}O_6 \sim O_7$, which deemed me the substance was xanthophyll on the way of oxidation.

(5) "Yellow cocoons' odor".

Xanthophylls both on the way of oxidation and after being utterly oxidized have a violet-like odor, peculiar to the yellow cocoon. Depression of the melting point and increase in volatility by the oxidation of xanthophyll elucidate its smelling power greatly.

On the Chemical Constituents of Rice-embryo.

By

Riang-Ha KIMM and (the late) Taro NOGUCHI.

(Received March 1, 1933.)

Rice-embryo constitutes only 3% by weight of the whole grain and it is very difficult to separate from it in a pure state in large quantities, so its chemical composition has not yet been studied thoroughly.

In 1912, Luigi Bernardini(1) reported on the analysis of the ash and the distribution of phosphoric acid in different forms in the embryo and he pointed out that the embryo is very rich in phosphoric acid of which the greater part is in the phytin-form.

M. Hamada(2) isolated protein from the embryo, determined the distribution of amino acids in its hydrolytic products and demonstrated by feeding experiment that the protein in the embryo has a very high nutritive value. Later, Hirai(3) investigated the ethersoluble substances of the embryo and isolated palmitic, oleic and linolic acids as the chief constituents of fats. Further, from the unsaponifiable fraction, he obtained the so-called Burian's phytosterol, besides a mixture of sterols which was readily soluble in organic solvents. From its high melting point (144°C) and low rotatory power (−23°) it was assumed by him to contain a dihydrositosterol.

Quite recently, the rice-polishing factories, Munechika and Niko & Co. have independently invented new polishing machines by which the embryo can be easily separated from the grain in an intact state and large quantities of these samples were kindly supplied by these factories to the present authors for investigation.

These samples, when carefully refined, looked quite homogeneous and were absolutely free from crushed grains, brans or other impurities. They contained in average 24% fatty matter, including about 1.2% unsaponifiable substances, besides a little waxes and a phytosterine-glucoside (phytosterolin). The waxes chiefly consisted of melissyl cerotate. Of the fatty acids, 25% were saturated and 75% unsaturated acids. From the saturated acids, palmitic, arachidic, and cerotic acids were isolated and identified; further, the presence of a little myristic and stearic acids were proved. The unsaturated acids consisted almost exclusively of oleic and linolic acids. The absence of other higher unsaturated acids was confirmed by the examination of the bromination products.

The unsaponifiable matter was treated with aceton and absolute alcohol and separated into two parts, i. e. a white amorphous powder (80%) and a

brown viscous syrup (20%). The former gave strong Liebermann reaction for sterine and the characteristic reaction for ergosterine. By repeated recrystallization from various organic solvents, some melissyl alcohol, a small amount of dihydrositosterine, stigmasterine and the so-called Anderson's γ-sitosterine[4] were isolated and identified. The existence of a little ergosterine was confirmed by converting it into vitamin D by irradiation with ultraviolet rays. Futher, the mother liquor of the above substances was fractionally crystallized from hexane and a new sitosterine (m. p. 156°C, $[\alpha]_D = -14.49°$) was isolated. It was converted into an acetate (m. p. 111°C, $[\alpha]_D = -9.68°$) and a benzoate (m. p. 129°C, $[\alpha]_D = +14.48°$). This new sterine is comparatively soluble in light petroleum ether and more readily in hexane and other organic solvents. The so-called Anderson's α-sitosterine[4], which was presumed by him to exist in rice polishing oil, is most probably the mixture of various sterines.

A cerebroside-like substance was also detected in the ether extract of the embryo. Probably it is the same as that obtained by W. Trier[5] from rice grain. The nature of this substance will be investigated later on.

The authors have thus confirmed that the composition of the embryo oil closely resembles that of the polishing oil,

Experimental.

(I) *General composition of rice-embryo.*

The composition of the purified embryo, supplied from tne Niko & Co., is compared with that reported by each of Sawamura and Hamada in the following table:

	Present authors	Sawamura	Hamada
Moisture	9 83	5.73	10.41
Crude protein	21.26	24.30	20.76
Protein	18.10	——	17.56
Crude fat	23.87	21.05	20.66
Fibre	3.48	9.77	10.11
N-free extract	33.76	25.68	27.82
Ash	9.09	13.47	10.24
Pentosan	5.—	——	——
Carbohydrate	21.—	13.—	——

The composition of the ash is compared with that of Bernardini[1] as follows:

	Present authors	Bernardini		Present authors	Bernardini
P_2O_5	5.020	6.200	MgO	1.104	1.389
SiO_2	0.370	0.250	Mn_3O_4	0.012	trace
Fe_2O_3	0.064	0.060	K_2O	2.022	1.691
CaO	0.312	0.279	Na_2O	0.196	trace

Bernardini moreover determined the distribution of phosphoric acid in the embryo and in the whole grain, with the following results:

P_2O_5 in form of	Embryo		Seed	
	In 100 parts of dry matter	In 100 parts of total P_2O_5	In 100 parts of dry matter	In 100 parts of total F_2O_5
Lecithin	0.04	0.64	0.003	0.35
Phosphatides	0.22	3.54	0.018	1.86
Phytin	5.14	82.90	0.436	45.68
Inōrganic salts	0.04	0.64	trace	——
Nucleic acids	0.76	12.28	0.502	52.61
Total	6.20	100.00	0.950	100.00

The above table shows that the embryo is very rich in phospooric acid of which the greater part is in the phytin-form.

(II) *Chemical properties of the embryo oil.*

The chemical constants of the embryo oil, extracted from the above sample with ether, are compared with the result obtained by Hirai[5] and with that of polishing oil, reported by Takahashi[6]:

	Embryo oil		Polishing oil
	Present authors	Hirai	Takahashi
Acid value	26.5	29.74	14.36
Saponification value	183.0	212.19	183.54
Acetyl value	10.6	——	——
Iodine value	114.8	106.63	104.73
Hehner value	93.2	95.16	95.95
Reichert-Wollny value	0.4	2 05	0.73
Unsaponifiable matter	5.28%	4.00%	3.40%

The fatty acids obtained by saponifying the embryo oil with alcoholic potash in the usual way had the following constants:

m. p. 31～34°C; Acid value 196.3; Iodine value 109.9; Mean molecular weight 283; Liquid fatty acids 75%.

(III) *Phytosterolin (phytosterine-glucoside).*

When the etherial extract of the rice-embryo was left standing for several hours, a brown precipitate separated out, which when recrystallized from amyl alcohol formed white needles melting at 283～290°C and gave the Liebermann's sterine reaction as well as the Molisch's reaction for sugar. From the analysis as well as from other properties, this substance was proved to be identical with the phytosterolin isolated from wheat-embryo by Nakamura and Ichiba[7].

Analysis of the phytosterolin from rice-embryo:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.752	10.005	3.464	72.73	10.33
II	3.711	9.847	3.415	72.37	10.29
Calculated for phytosterolin ($C_{33}H_{53}O_6$)				72.30	10.20

Like other sterines, this substance forms an acetate melting at 167～168°C.

Analysis of the acetate:

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
4.378	11.059	3.503	68.90	8.95
Calculated for $C_{14}H_{64}O_{10}$			68.67	6.94

It forms also a benzoate melting at 198°C.

Analysis of the benzoate:

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
3.131	8.726	2.169	76.01	7.75
Calculated for $C_{61}H_{72}O_{10}$			75.90	7.52

(IV) *Separation of saturated fatty acids.*

Rice-embryo (1.5 kg) was boiled with 3.5 litres of 90% alcohol for three hours, filtered while hot and the residue was extracted three times each with 2.5 litres of 90% alcohol. The combined extract was allowed to stand overnight, whereby a flocky precipitate separated out which chiefly consisted of waxes. It was filtered off and the filtrate was evaporated in vacuum and the residue was extracted with ether. The etherial extract was, after evaporating off the ether, saponified with alcoholic potash according to the usual method. From the mixed fatty acids thus obtained the solid saturated acids were separated by Tortelli Ruggeri's method. They had the following properties:

m. p. 60～61°C; Mean molecular weight 270; Acid value 208; Iodine value 5.

The solid acids were now mixed with sand and dried on a waterbath for ten days and extracted with ether. The etherial solution was evaporated,

the residue was again dissolved in petroleum ether, filtered, and the filtrate was evaporated, whereby, 100 g saturated acids were obtained.

For the isolation of each fatty acid, the above acid mixture was converted into methyl ester and subjected to fractional distillation. For this purpose the mixture was dissolved in 220 cc methyl alcohol, treated with dry HCl gas until the solution absorbed about 8 g of the acid and after heating for two hours on a water-bath the solution was shaken with ether. The etherial extract was, after washing with water to remove the methyl alcohol and hydrochloric acid, fractionally distilled under diminished pressure (8 mm). In this way the following fractions were obtained:

Fraction	Temperature (°C)	Yield (g)
I	160~176	1.5
II	176~186	80.5
III	186~213	7.2
IV (residue)	——	15.5

Each fraction was now saponified and free fatty acids thus regenerated had the following constants:

	Yield (g)	Acid value	Mean mol. wt.	m. p. (°C)
I	1.2	220.6	254.2	53~54
II	75.0	216 8	258 7	58~59
III	6 5	212 8	263.6	59~60
IV	12.3	——	——	——

The fraction I was recrystallized from 90% alcohol and the residue from the mother liquor, after evaporating the alcohol, was recrystallized twice from 80% alcohol. Again, the residuals from the combined mother liquors were recyrstallized twice from 70% alcohol. Then, evaporating off the combined mother liquors, the residual was dissolved in hot 70% alcohol, decolorized with animal charcoal and allowed to stand overnight. Thereupon, a crystalline precipitate separated out which melted at 54°C and had the mean molecular weight 234. Most probably it was myristic acid containing some impurities.

The fraction II, when recrystallized four times from 95% alcohol, melted at 62~63°C and had the acid value 218.8 and the mean molecular weight 256 3. The analysis gave the following results:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	4.315	11.900	4.853	75.22	12.58
II	4.001	11.000	4.487	74.98	12.55
	Calculated for $C_{18}H_{32}O_2$			74.92	12.59

The analysis shows that it was palmitic acid.

The fraction III was coloured slightly yellowish, so it was dissolved in alcohol and after being decolorized with animal charcoal, recrystallized fifteen times from absolute alcohol. The crystals thus obtained melted at 59~61°C and had the acid value 197.8 and the mean molecular weight 282. It was proved to be a mixture of stearic and palmitic acids, but the yield was too small for further purification.

The fraction IV was dissolved in 500 cc absolute alcohol and allowed to stand for 48 hours. The precipitate formed thereby was collected and washed with absolute alcohol: the yield, 3.4 g. After recrystallization from absolute alcohol eight times more, the crystals obtained melted at 76.5~77.5°C with the acid value 141.1 and the mean molecular weight 398.2. The analysis gave the following results

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3 903	11 209	4 533	78 33	12 99
II	3 587	10 301	4 250	78 33	13 25
	Calculated for $C_{26}H_{52}O_2$			78 61	13 13

The analysis shows the agreement with cerotic acid The filtrates of cerotic acid were united, evaporated and the residue was recrystallized three times from absolute alcohol. The fatty acid obtained in this way had the melting point 76°C and the mean molecular weight 318. From this it was concluded that arachidic acid was present; but it could not be purified.

The mother liquor of fraction IV was diluted to 90% alcohol, the precipitate formed thereby was dissolved by heating and allowed to stand for three days, when the precipitate was collected, recrystallized from 90% alcohol and the filtrate therefrom was evaporated and the residue was recrystallized from 90% alcohol five times. The fatty acid thus obtained was long needles melting at 79.5~80°C with the acid value 164 and the mean molecular weight 342.

Analysis of the fatty acid:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	4 374	12 429	5 121	77 50	13 10
II	4 942	14 031	5 716	77 43	12 94
	Calculated for $C_{22}H_{44}O_2$			77 57	13 03

The analysis shows the substance to agree with behenic acid.

(V) *Bromination of unsaturated fatty acids.*

Unsaturated fatty acids (150 g) were brominated in usual way and the bromination products were digested with 2 L of boiling petroleum ether (35~55°C), whereby a clear solution was obtained leaving no insoluble residue.

On standing overnight, 73 g of solid bromides separated out. The mother liquor, when concentrated to 1/3 of its volume and allowed to stand for three weeks, gave 3 g more of solid bromides.

When the solid bromide thus obtained was recrystallized three times from 95% alcohol, it melted at 113°C and had the acid value 93.7 and the mean molecular weight 599.9.

Analysis of the soild bromide:

	Snbstance (mg)	AgBr (mg)	Br (%)
I	6.645	8.295	53.12
II	6.030	7.553	53.30
	Calculated for $C_{18}H_{32}O_2Br_4$		53.33

The analysis shows it to agree with the tetrabromide of linolic acid.

When the filtrate of the solid bromide was evaporated, another bromide having 41.3% bromine was obtained. Probably it was the dibromide of oleic acid mixed with some tetrabromide.

(VI) *Oxidation products of the unsaturated fatty acids.*

When the unsaturated fatty acids were oxidized with alkaline premanganate according to the Hazura's method, 11.3 g insoluble hydroxyacids were obtained. They were treated with ether and separated into the ether-soluble and insoluble parts. The former, when recrystallized from absolute alcohol, melted at 131°C with the acid value 176.3 and the mean molecular weight 316.7. This was proved to be dihydroxystearic acid.

Analysis of the dihydoxystearic acid:

Substance (g)	CO_2 (g)	H_2O (g)	C (%)	H (%)
0.0982	0.2462	0.09852	68.35	11.14
	Calculated for $C_{18}H_{36}C_4$		68.29	11.47

The ether-insoluble part, when recrystallized from absolute alcohol, melted at 173°C, its acid value being 160.9 and the mean molecular weight, 394.1. It was sativic acid.

Analysis of the sativic acid:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.352	7.620	3.036	62.00	10.13
II	3.611	8.178	3.391	62.07	10.35
	Calculated for $C_{18}H_{36}O_6$			62.07	10.35

Linusic or isolinusic acid could not be detected.

(VII) *Unsaponifiable matter.*

When 20 kg rice.embryo was extracted with benzene, about 4 kg dark brown oil was obtained. It was dissolved in 12 L ether, a little insoluble waxy substance was filtered off and evaporated. The oil thus obtained was saponified with alcoholic potash in the usual way and 150 g of an uusaponifiable

substance were obtained. It was a light brown semi-solid mass having a characteristic odour and giving a strong Liebermann-Buchard's reaction as well as the colour reaction of ergosterine. By treating with acetone and absolute alcohol it was separated into two part, i. e. (A) a white amorphous solid (120 g) and (B) a brown syrup (30 g).

(A) From the spectrographic studies it was calculated that the amorphous solid contained about 0.4～0.5% ergosterine. When irradiated with ultra-violet rays and tested on rats, the existence of vitamin D was clearly demonstrated.

(1) Melissyl alcohol.

When the amorphous solid (A), mentioned above, was recrystallized several times from absolute alcohol until it gave no more Liebermann-Buchard's reaction, about 0.05 g crystals was obtained which melted at 83°C.

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
2.798	6.462	3.524	82.48	14.09
	Calculated for $C_{30}H_{62}O$		82.19	14.15

The analysis shows it to agree with melissyl alcohol.

(2) Dihydrositosterol.

The mother liquors of the last few fraction of the said recrystallization were combined and evaporated. About 30 g of the residue thus obtained were again repeatedly recrystallized from absolute alcohol, and after drying, it weighed 3.2 g; m. p. 132°C, $[\alpha]_D^{22} = -6.20°$. The unsaturated sterines were decomposed by the Anderson's method, and about 0.13 g dihydrositosterine (m. p. 144°C, $[\alpha]_D^{22} = +24.28°$) was isolated and identifiedl.

The sterol obtained from the combined filtrate of the above recrystallization melted at 138～140°C, $[\alpha]_D^{23} = -26°$. This was extracted three times with a large quantity of petroleum ether (50°C) and then it was converted into acetate; m. p. 115～11°C, $[\alpha]_D^{22} = -23.98°$.

(3) Tetrabromide of stigmasterine acetate.

When the acetate, mentioned above, was recrystallized ten times from absolute alcohol, the top fraction (12 g) melted at 138°C and had the rotation, $[\alpha]_D^{19} = -43.7°$. This fraction was brominated by the Windaus-Hauth method adding an excess of glacial acetic acid. The precipitate was collected on a filter, dissolved again in chloroform and thrown down by methyl alcohol; m. p. 204～205°C.

Analysis of the bromide:

	Substance (mg)	AgBr (mg)	Br (%)
I	5.235	4.970	40.40
II	8.330	8.030	41.00
	Calculated for $C_{32}H_{52}O_2Br_4$		40.58

The above result agrees with the tetrabromide of stigmasterine acetate.

Owing to the scarcity of the material, the free stigmasterine could not be obtained.

(4) Anderson's γ-sitosterine[4].

The bromides in the mother liquor of the above tetrabromide were combined, dissolved in ether and fractionally precipitated with methyl alcohol. The main fraction thus obtained was an amorphous powder, melting at 130°C. It was debrominated by boiling with zinc dust and acetic acid, and an acetyl derivative (m. p. 143°C, $[\alpha]_D^{20} = -44.70°$) was prepared. By saponifying the latter, a free sterol (m. p. 148°C, $[\alpha]_D^{21} = -42.3°$) was isolated.

Analysis of the free sterol:

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
4.013	12.270	3.950	83.39	12.30
	Calculated for $C_{29}H_{50}O$		83.96	12.24

The analysis shows that it agrees with Anderson' γ-sitosterol.

(5) A new sitosterol.

The sterol acetates descrbied above were combined and saponified. About 80 g free sterol thus obtained, was dissolved in hexane and fractionally precipitated by gradual cooling as follows:

	Temperature (°C)	Yield (g)	m. p. (°C)	D
I	21	19.5	135	−29 34° (21°C)
II	1	16.5	134	−23.93° (20°C)
III	−12	20.0	132	−18.46° (18°C)
IV (residue)		22.0	148	——

The author could not separate from the above fraction I, II, III any substance that had constant melting point and rotation. The last fraction (IV) was recrystallized from absolute alcohol ten times, from acetone twice and further from methyl alcohol fifteen times and a new sitosterol (m. p. 156°C, $[\alpha]_D^{23} = -14.485°$) was isolated; yield, 2.1 g. It was more readily soluble in organic solvents than other sterines.

Analysis of the new sitosterol:

	Substace (mg)	Co_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.269	10.040	3.495	83.76	11.96
II	4.144	12.690	4.510	83.52	12.18
		Calculated for $C_{27}H_{46}O$		83.85	12.01

The acetate of the new sitosterol was prepared by boiling the free sterol with acetic anhydride for 40 minutes in the usual way; m. p. 111°C, $[\alpha]_D^{23} = -9.87°$.

Analysis of the acetate:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.472	10.370	3.608	81.46	11.63
II	3.849	11.467	3.949	81.25	11.48
	Calculated for $C_{29}H_{48}O_2$			81.21	11.30

The propionate was obtained by boiling the free sterol with propionic anhydride for 1.5 hours in the usual way; m. p. 106°C, $[\alpha]_D^{23} = -6.78°$

Analysis of the propionate:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	3.003	8.992	3.177	81.66	11.84
II	3.610	10 800	3.750	81 59	11 62
	Calculated for $C_{30}H_{50}O_2$			81.74	11.60

The benzoate was obtained by adding benzoylchloride to the sterol dissolved in pyridine at 2°C; m. p. 129°C, $[\alpha]_D^{20} = +14.48°$.

Analysis of the benzoate:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	2 981	9 071	2.885	82 99	10.83
II	3 660	11.178	3 577	83 29	10.93
	Calculated for $C_{34}H_{54}O_2$			83 21	10.28

The acetate mentioned above was brominated by the Windaus-Hauth method, but when a small amount of bromine was added, the solution became brownish green, liberating HBr and required 50% more bromine than the calculated quantity. When bromine was added at −13°C, the green colour never appeared, only turning brown. The percentage of bromine was found to be 31.89 and 32.27%. A portion of the sterol was reduced with hydrogen using platinum-black as the catalyst, but the reduction proceeded very slowly, so that the Liebermann-Buchard reaction never disappeared. The molecular formula of the sterol calculated from its analytical results corresponds to $C_{27}H_{46}O$.

From the above observation it was believed to be an isomer of Burian's sitosterol. The so-called Anderson's α-sitosterol was still a mixture of various sterols, as is shown in the following table:

	Free sterol		Acetate	
	m p. (°C)	Rotation	m. p. (°C)	Rotation
α-Sitosterol	138~140	−23.41°	115~116	−23.91°
Author's new sterol	156	−14.485°	111	− 9.87°

(B) The brown syrup.

The brown syrup was subjected to steam distillation and a little oil having a characteristic odour was obtained, but the amount was so small that it could not be examined further. From the residue, when further distilled

at high vacuum a hydrocarbon mixed with a little higher alcohol was obtained.

(VIII) *Wax.*

When rice-embryo was boiled with strong alcohol or benzene, then filtered while hot and the filtrate was left to stand for several hours, the waxy substance separated out as a greyish white flocky precipitate, or when the crude embryo oil was treated with ether, the waxy substance remained as an insoluble residue. These were combined and extracted with hot benzene in Soxhlet apparatus and the extract was precipitated with ether. By repeating this operation five times needle-shaped crystals melting at 82°C were obtained; yield, 3.2 g.

The analysis gave the following results:

	Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I	4.000	12 055	4 954	82 19	13.86
II	3.840	11.642	4 858	82 17	14.07
		Calculated for $C_{55}H_{112}O_2$		82.06	14.03

The analysis shows that it was melissyl cerotate.

For further identification, this substance was saponified by boilling with 5% potassium alcoholate for 4 hours. After cooling, the mixture was treated with alcoholic $CaCl_2$. The calcium soap thus formed was filtered off, and the filtrate was completely dried and extracted with benzene. The benzene solution was evaporated and the residue was recrystallized from absolute alcohol. In this way hair-like needles melting at 85°C were obtained.

Analysis of the sample:

Substance (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
4.100	12.400	5 230	82.48	14.27
	Calculated for $C_{30}H_{62}O$		82.08	14.28

The result agrees with melissyl alcohol.

The Ca-soap obtained as above, was decomposed with dilute hydrochloric acid (1 : 1) and cerotic acid was isolated as insoluble crystalline powder. After recrystallization from absolute alcohol, it melted at 78°C and had the mean molecular weight 394. Mixed with a pure specimen of cerotic acid, no depression of melting point was observed.

(XI) *Cerebroside.*

When rice-embryo, previously extracted with benzol, was boiled with 85 ~90% methyl alcohol and the methyl alcoholic solution was evaporated in vacuum to a syrupy consistency and left standing for several days, a solid substance separated out. It was collected by suction and dissolved in ether. The etherial solution thus obtained was, after washing with water, treated with acetone, whereby a precipitate was formed which gave ninhydrine and

Molisch's reactions. This precipirate was dissolved in ether and precipitated by adding absolute alcohol. By repeating this operation four times, a crystalline substance was obtained which gave no more the above reactions. When dried in vacuum desiccator it became brownish black and was no more soluble in ether. The mother liquor of the above crystals was shaken with water and the separated ether layer was dehydrated, evaporated to a small volume and treated with acetone, whereby a precipitate was formed, which when recrystallized from absolute alcohol gave ninhydrine and Molish's reactions. It resembled the cerebroside, obtained by Trier from rice grain. Owing to the scarcity of the material, it was not further investigated. Details concerning this part will be published later.

In conclusion, the authors wish to express their sincere thanks to Prof. Dr. U. Suzuki for his advices and encouragement throughout this work and also to Mr. T. Iki and T. Tanaka for micro-analysis.

Studies on the Fermentation products by Mould Fungi. Part X.

Glaucic Acid formed by Aspergillus glaucus. (Aspergillus glaucus. IV.)

By

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(Received May 23, 1933).

On the previous report of this series (part IV), the author described that a new crystal substance, m. pt. 238∼9°, was obtained by the cultivation of *Aspergillus glaucus* under a certain condition. Since, the author cultivated *Aspergillus glaucus* several times under the same condition but could not obtain this crystal substance. Unexpectedly, the author obtained this substance by treating the mycelium of *Aspergillus glaucus* with ether on account of the isolation of chitin from the mycelium. This new crystal substance was named glaucic acid after *Aspergillus glaucus*.

Glaucic acid melts at 238∼9° and contains no nitrogen, methoxyl, ketomethylen and methylenoxide groups. The results of analyses of glaucic acid and its derivative show that the molecular formula of glaucic acid is $C_{17}H_{22}O_6$.

The existence of one carboxyl group in the glaucic acid is identified by the following experiments. Glaucic acid is soluble in Na-carbonate and precipitated by the addition of mineral acid. The results of titration with standard alkali, using phenolphthalein as the indicator, shows glaucic acid is a monobasic acid. Moreover glaucic acid is not methylated with dimethylsulphate but methylated with diazomethane and this monomethylderivative is insoluble in Na-carbonate and alkali.

By treating glaucic acid with benzoylchloride or acetic acid anhydride, the benzoyl- or acetyl-derivatives are not obtained. Moreover, the results of quantitative determination of hydroxyl group in glaucic acid by the method of Tschugaeff and Zerewitinoff shows the existence of one hydroxyl group and this fact indorses the existence of one carboxyl group.

Isolation and properties of glaucic acid.

The mycelium of Aspergillus glaucus grown on the medium which contains glucose, peptone and mineral matter is washed with water completely, dried at 100°, powdered and extracted with ether for a week. On evaporating off the solvent from the extract, glaucic acid crystallizes in prism. Yield : 1.0 g. of glaucic acid from 411 g. of mycelium. This crude substance is dissolved in dilute alkali, precipitated with hydrochloric acid, washed with hot water and then recrystallized from boiling alcohol. Glaucic acid, prisms, melts at 238～9° and contains no nitrogen. The reactions of methoxyl group by micro-Zeisel, methylenoxide group by Tollen's phloroglucinol and ketomethylen group by Na-nitroprusside are all negative. It is soluble in Na-carbonate, alkali and precipitated by the addition of mineral acid. It is also easily soluble in boiling ethyl-, methyl-alcohols, acetone, difficultly in benzene, chloroform, acetic ester and nearly insoluble in boiling water.

Subst. (mg.)	CO_2 (mg.)	H_2O (mg.)	C %	H%
3.218	8.019	2.182	66.92	7.47
3.142	7.679	2.182	66.65	7.77
3.358	8.193	2.228	66.54	7.42

(g.)	Campher (g.)	ΔT (°)	M.W.
0.0110	0.1130	13	300
0.0133	0.1335	13	306
0.1129	$n/10$ NaOH 3.515 ccm.		304
0.0974	CH_4 5.6 ccm. (762.5 mm, 20°)		

	M.W.	C %	H%	O%	OH%
$C_{17}H_{22}O_5$ cal.	306	66.67	7.19	26.14	5.56
($C_{16}H_{21}O_3$ COOH) fou. (average)	303	66.70	7.42	25.74	4.39

Glaucic acid monomethyl ester : — 0.3 g. of glaucic acid is suspended in ether and added with ethereal solution of diazomethane prepared from 3 ccm. of nitrosomethylurethane. Yield : 0.31 g. The methylester is easily soluble in acetic ester, benzene, chloroform, difficultily in ethylalcohol, ligroine and in-

soluble in water, Na-carbonate and alkali. It is recrystallized from alcohol or ligroin. Needles. M. pt. 188~90°.

Subst. (mg.) 3.200	CO_2 (mg.) 7.873		H_2O (mg.) 2.234
〃 4.040	AgJ 2.673		
	C %	H%	OCH_3%
$C_{17}H_{21}O_4(OCH_3)$ cal.	67.50	7.50	9.69
foun.	67.10	7.80	8.74

Methylation with dimethylsulphate : — 0.2 g. of glaucic acid is dissolved in 40 ccm. of methylalcohol and shaked vigorously for 20 minutes adding 1 ccm. of dimethylsulphate and 2 ccm. of 25% kaliumhydroxide. After repeating this procedure 5 times, the reaction mixture is evaporated, acidified and filtered. Yield : 0.15 g. It is recrystallized from benzene. Prisms. M. pt. 234~5°. The admixture with glaucic acid melts at 236~7°.

Subst. (mg.)	CO_2 (mg.)	H_2O (mg.)
3.394	8.218	2.270
	C %	H %
$C_{17}H_{22}O_5$ cal.	66.67	7.19
foun.	66.40	7.48

Acetylation and benzoylation : — 0.2 g. of glaucic acid is added with 5 g. of acetic acid anhydride and 0.5 g. of dehydrated Na-acetate and boiled for 4 hours under the reflux condenser in the oil bath. The reaction mixture is poured into water and the precipitate is filtered. Yield : 0.2 g. It is recrystallized from dilute methylalcohol. Prisms. M. pt. 234~5°. The admixture with glaucic acid also melts at 235~6°.

0.2 g. of glaucic acid is dissolved in pyridine and added with 0.4 g. of benzoylchloride. After several days, the reaction mixture is poured into water and the precipitate occured is filtered. Yeild : 0.2 g. It is recrystallized from alcohol. Prisms. M. pt. 234~5°. The m. pt. of admixture with glaucic acid shows no depresssion.

Biochemistry of Filamentous Fungi. II.

A Metabolic Product of Aspergillus melleus Yukawa. Part I.

By

Hidejiro NISHIKAWA.

(Received May 31, 1933)

As is well known, filamentous fungi emit more or less peculiar mouldy

smell, differing in nuance and intensity according to species or varieties; some produce even agreeable odour, as, for instance, Aspergillus oryzae. The chemical nature of their smell has, however, been little investigated, nothing definite being known as it. It is presumably due to mixture of minute quantities of diverse substances and consequently it will in general be tedious task to isolate each constituent in sufficient quantity for chemical investigation. But researches in this line will provide some scientific interest and be not without significance for the physiology of mould fungi.

The author has isolated from the culture medium of Aspergillus melleus a crystalline substance which is supposed to be a constituent of the smell of the mould. It was provisionally named mellein. Analysis of mellein and its nitro-derivative show that its probable formula will be $C_{10}H_{10}O_3$. Purple coloration with $FeCl_3$, positive Millon's reaction, and precipitation with bromine water from aqueous solution indicate its phenolic character.

In 1931 and 1932 a substance, $C_{14}H_{14}O_4$, m.p. 58.5°, was isolated and described by Yamano[1] from cultural solution of Aspergillus ochraceus. This substance and mellein which melts at 58° have almost the same melting point ann their properties and reactions are quite similar. As Asp. melleus and ochraceus have close morphological resemblance and according to Thom and Church[2] Asp. melleus is to belong to Asp. ochraceus group, possible identity or at least strong similarity in structure of both substances could be anticipated.

Experimental.

Preparation of mellein.

The strain of Aspergillus melleus used in the present investigation was purchased from the Centraalbureau voor Schimmelcultures, Baarn, Holland. The culture medium was of the following composition: —

Sucrose (commercial)	50 g.	$MgSO_4 \cdot 7H_2O$	0.5 g.
$NaNO_3$	2 g.	$FeSO_4 \cdot 7H_2O$	0.01 g.
KH_2PO_4	1 g.	H_2O	1000 c.c.
KCl	0 5 g.		

The above medium contained in Erlenmeyer flasks was sterilized and conidia of Asp. melleus were abundantly inoculated in it. After 2 to 3 weeks of incubation at 30° the medium was filtered free from mycelial felt, saturated with common salt and extracted with ether. The ether extractives were then submitted to steam distillation and the distillate was again extracted with ether. When ether was driven off, the residue solidified to crystalline magma. Yield, 0.3 g. from 1 litre of the medium, corresponding to 0.6 per cent. of the sugar used. This is already fairly pure. The specimen purified by disolving in alkali and reprecipitating with acid was submitted to analysis.

Anal. Subst. $=0.1850$; $CO_2=0.4578$; $H_2O=0.0968$ g.

Found : $C=67.49$; $H=5.81\%$

Calc. for $C_{10}H_{10}O_3$: $C=67.42$: $H=5.62\%$.

Mol. wt. 0 2054 g. subst. in 21.98 g. benzene. (Cryoscopy).

$d=0.281°$, $M=162.4$. $C_{10}H_{10}O_3=178$

Rotatory power. 0.1988 g. subst. in 25 c.c, chloroform.

$\alpha_D^{12}=-0.86°$. $[\alpha]_D^{12}=-108.15°$.

Mellein crystallizes in colourless long prisms, sparingly soluble in water, soluble in ethyl and methyl alcohol, chloroform, ether, and light petroleum. Purple coloration with $FeCl_3$ is marked in 1/10,000, faintly visible even in 1/100,000 dilution. From solution in alkali mellein is precipitated by passing CO_2.

Nitration of mellein:— Mellein was dissolved in conc. nitric acid and stood over nicht. On addition of water white flocky precipitate separated out. Pale yellow beautiful needles with greenish tint were obtained when recrystallized from methyl alcohol.

Anal. Subst. $=0.1720$; $CO_2=0.3404$; $H_2O=0.0652$ g.

Subst. $=0.2276$; $N_2=12.8$ c.c. (19.5°, 766 mm).

Found: $C=53.97$; $H=4.21$; $N=6.47\%$.

Calc. for $C_{10}H_9O_5N$: $C=53.81$; $H=4.04$; $N=6.28\%$.

Rotatory power. 0.1996 g. subst. in 25 c.c. Chloroform.

$\alpha_D^{19}=-1.37°$. $[\alpha]_D^{19}=-171.59°$.

Analysis agree with a mononitro-derivative af mellein. It melts at 183 ~184° after turned yellow in the neighbourhood of 140°. It is insoluble in ether and light petroleum, sparingly soluble in water, soluble in benzene, methyl and ethyl alcohol, readily soluble in acetone and chloroform. It gives an intensely yellow solution when dissolved in alkali and separates out as white precipitate by acid. Its aqueous solution turns light red, ethyl alcoholic solution blood red on addition of $FeCl_3$.

References.

(1) Yamano: Jôzôgaku Zasshi, Vol. 9, p. 514, (1931).
  〃 Jôzôgaku Zasshi, Vol. 10, p. 528, (1932).

(2) Thom & Church: The Aspergilli, 1926, p. 73 & 188.

Studies on the Calcium and Magnesium Absorption of Rice Plants at Different Stages of their Growth in Water Culture.

By

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(Received June 26, 1933)

Résume.

Experiment on the calcium and magnesium absorption of rice plants at different stages of their growth was conducted with water culture as a means of elucidating their nutritional physiology. The conclusions obtained are as follows : —

(1) The presence of calcium in nutrient solution is absolutely necessary for rice plants till the 13th week after germination in order to assure their attaining maturity. After that time, the lack of calcium has no harmful effects on the growth, as seen by a comparison of plants under calcium-free nutrients with the control, which is supplied with complete nutrients through the entire period of growth. But the earlier the time of the calcium deficiency in nutrient solution within 13 weeks after germination, the more hindered was the growth of rice plants.

The presence of magnesium in nutrient solution is necessary and sufficient for the full growth of rice plants till the 7th week after germination. So their growth is not hindered by the lack of magnesium after that time.

W. F. Gericke concludes in his essay "the absence of one or more of elements calcium and magnesium after initial exposure of rice plants to the complete nutrient solution, did not have any pronounced harmful effect on the development." and in the case of calcium, his data show that the period of initial exposure is about 6 weeks ; but the writers experiment shows that, in the case of calcium, its presence for 13 weeks in nutrient solution is inevitably necessary for the growth of rice plants. In the case of magnesium, the writers result almost agrees with that of Gericke.

(2) The growth of rice plants is not affected by the lack of calcium in nutrient solution till the 5th week after germination, if there is a supply of calcium after that time. But the earlier the time of supply, the better the recovery of the growth which is badly influenced by calcium deficiency. When a supply of calcium was lacking for more than 6 weeks after germi-

nation, the growth could no more recover in spite of giving a supply of calcium. In most serious cases, plants died.

The lack of magnesium till the 6th week after germination dose not affect the development of the plants except for a little ill effect on seed formation. However, the earlier the time of magnesium supply, the better the growth of plants.

(3) As the presence of calcium is necessary for the development of rice plants through almost all stages of growth, it is hard to show when calcium is utilized most significantly. But it is supposed to be from the 5th to the 7th week after germination.

In the case of magnesium, the maximum point of utilization is supposed to lie between the 4th and 8th week after germination.

(4) The ratio of CaO to MgO absoibed by rice plants is about 3 : 1 in weight at an early period of their growth ; it gradually approaches to 1 : 1. However, the ratio of CaO to MgO absorbed by plants in the entire period of their growth is 1.2 : 1.0 in weight.

Sterilizing Action of Acids. IV Report.

Sterilizing Action of Unsaturated Monobasic Fatty Acids on Putrifactive bacteria, Bac. typhosus, and Vib. cholerae.

By

Sogo TETSUMOTO.

(Received June 14, 1933).

Contents

(1) Reagents.

Details of the sterilizing action of saturated monobasic fatty acids $C_nH_{2n+1}CO_2H$ are as reported before.

To know the sterilizing action of unsaturated monybasic fatty acids on bacteria by comparing it with the action of the saturated fatty acid series, I used the following acids.

Table I.—Reagents. $C_nH_{2n-2}CO_2H$.

Acids	numbeo of C atom	Rational formulae	Molecular weight	PH at N/100	PH at N/1000
Acrylic acid	C_3	$CH_2:CH \cdot CO_2H$	72.047	3.13	3 63
nor. Crotonic acid	C_4	$CH_2:CH \cdot CH_2 \cdot CO_2H$	86.068	3.33	3.83
Undecylenic acid	C_{11}	$CH_2 \cdot CH \cdot (CH_2)_8 \cdot CH_2H$	184.071	saturated aq. solution	5.4
Oleic acid	C_{18}	$CH_3 \cdot (CH_2)_7CH:CH(CH)_7CO_2H$	282.362	〃	6.0

acrylic acid·· ···············made by Fraenkel & Landau Co.

other 3 acids·············· S. Kahlbaum Co.

Undecylenic acid and oleic acid are scarcely soluble in water, so I made saturated aqueous solution of these acids at 20°C.

(2) Experimental methods.

Experimental methods and used microorganisms and e.t.c. are the same as I reported before(1)(2).

(3) Sterilizing action at the same molecular concentration.

To know the sterilizing action at the same molecular concentration of unsaturated monobasic fatty acids on Staph. c. pyogen. aureus, Proteus vulgar., Hauser, Bac. typhosus and Vib. cholerae, I studied the sterilizing action of *N*/100 and *N*/1000 solutions of acrylic acid and normal crotonic acid, and saturated aqueous solution of undecylenic acid and oleic acid at 20°C. Results are as shown in the following table.

Table 2.—Sterilizing action at *N*/100.

Number of C atom	acids	pH	Surviving period															
			Staph. c pyog.				Prot vulgar.				Bac. typhosus				Vib. choler			
			h 3	6	9	12	m 60	90	h 2	3	m 60	90	h 2	3	m 5	10	15	20
C_3	Acrylic	3.1	+	+	−	−	+	+	−	−	−	−	±	−	+	±	−	−
C_4	nor. Crotonic	3.3	+	+	±	−	+	+	±	−	+	+	+	−	+	+	+	−
Control			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

m ··· minut, h ·· ·· · hour, +··· ·····alive, −······ ·· perished,

± · sometimes alive and sometimes perished.

Table 3.—Sterilizing action at *N*/1000 and saturated aqueous solution.

Number of C atom	Acids	pH	Surviving period																											
			Staph. c. pyogen							Prot. vulgar.							Bac. typhosus							Vib. choler.						
			m 60	90	h 12	24	36	d 5	6	m 30	45	h 9	12	d 2	3	4	m 45	60	h 12	24	36	d 3	4	m 1	2.5	45	60	90	h 2	3
C_3	Acrylic	3.6	+	+	+	−	−	−	−	+	+	+	−	−	−	−	+	+	+	−	−	−	−	+	+	±	−	−	−	−

C_4	Crotnic	3.8	+	+	+	+	−	−	−	+	+	+	±	−	−	−	+	+	+	±	−	−	−	+	+	+	−	−	−	−
C_{11}	Undecy-lenic	5.4	+	−	−	−	−	−	−	±	−	−	−	−	−	−	±	−	−	−	−	−	−	±	−	−	−	−	−	−
C_{18}	Oleic	6.0	+	+	+	+	+	±	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	±	−	−
	Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

d……day.

From results noted in Table 2 and 3, we find the following facts. At the same molecular concentration of unsaturated monobasic fatty acids, acrylic acid (C_3) is stronger than crotonic acid (C_4) as concerns to the sterilizing action. In the saturated aqueous solution at 20°C, undecylenic acid (C_{11}) has been dissolved in an extraordinarily small quantity but its sterilizing action is extremely strong. The sterilizing action of oleic (C_{18}) is very weak.

(4) Sterilizing action of anions of unsaturated monobasic fatty acids.

To know the sterilizing action of anions of each acid, I made neutral salts of Na, K, Ca and NH_4 having the same anions of each acid. Concentration of salts of acrylic acid and crotonic acid are $N/100$. And salts of undecylenic acid and oleic acid are in saturated aqueous solution at 20°C. respectively. The results are as shown in Table 4. The results of K salts are nearly the same as Na salts, so I denote the results of Na salts only.

Table 4.—Sterilizing action of anions of unsaturated monobasic fatty acids.

(I)—Na salts.

Na–	Surviving Period																			
	Staph c pyogen.						Prot vulgar. H					Bac. typhosus					Vfb. choler			
	h 6	9	d 5	d 6	7	8	h 2	3	d 4	5	6	h 3	6	d 5	6	7	m 25	5	h 3	6
acrylate	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
crotonate	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
undecylenate	±	−	−	−	−	−	±	−	−	−	−	+	−	−	−	−	+	−	−	−
oleiate	+	+	+	−	−	−	+	+	+	−	−	+	+	±	−	−	+	+	+	−
Control	+	+	+	+	+	+	+	+	+	±	−	+	+	+	±	−	+	+	+	+

(II)—Ca salt.

Ca–	Surviving period																			
	Staph. c pyogen						Prot. vulgar. H.					Bac. typhosus					Vib. choler.			
	h 4	6	d 5	6	7	8	m 90	h 2	d 3	4	5	h 3	6	d 5	6	7	m 25	5	h 3	3
acrylate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
crotonate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
undecylenate	+	−	−	−	−	−	±	−	−	−	−	±	−	−	−	−	±	−	−	−
oleiate	+	+	+	+	−	−	−	+	+	+	−	+	+	±	−	−	+	+	±	−
Control	+	+	+	+	+	+	−	+	−	+	±	+	+	+	±	−	+	+	+	+

(III)—NH_4 salts.

NH_4-	Surviving Period																			
	Staph. c. pyogen						Prot. vulgar. H.					Bac. typhosus					Vib. choler.			
	h 9	12	d 6	7	9	10	h 3	6	d 5	6	7	h 6	9	d 6	7	8	m 2.5	5	h 6	9
acrylate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
crotonate	+	+	+	+	±	−	+	+	+	+	−	+	+	+	+	−	+	+	+	−
undecylenate	±	−	−	−	−	−	±	−	−	−	−	±	−	−	−	−	+	±	−	−
oleiate	+	+	+	−	−	−	+	+	+	−	−	+	+	±	−	−	+	+	±	−
Control	+	+	+	+	−	−	+	+	±	−	−	+	+	±	−	−	+	+	+	+

From the results shown in Table 4 (I), (II), (III), we find the following facts. Salts of acrylic acid (C_3) and crotonic acid (C_4) have no sterilizing action. Salts of undecylenic acid (C_{11}) have very strong sterilizing action. Salts of oleic acid (C_{18}) have very weak sterilizing action compared with the control.

These salts are all in neutral aqueous solution and have the same anions as acids. By these facts we know following facts.

Anions of acrylic acid (C_3) and crotonic acid (C_4) have no sterilizing action. Anion af undecylenic acid (C_{11}) has an extremely strong sterilizing action. Anion of oleic acid (C_{18}) has very weak sterilizing action.

Among salts of Na, K, Ca and NH_4, Ca salts have a somewhat short surviving period and NH_4 salts have a somewhat long surviving period for bacteria.

(5) Comparison of sterilizing action between saturated and unsaturated of monobasic fatty acids at the same molecular concentration.

I compared the sterilizing action of each acids having the same number of C atoms, at the same molecular concentration and in saturated aqueous solution at 20°C.

The results are as shown in the following table. (Table 5, (I), (II).)

Table 5.—Comparison of the sterilizing action between saturated and unsaturated monobasic fatty acids.

(I)—Results at *N*/100.

Number of C atom	Microorganisms		Staph. c. pyog.				Prot. vulgar. H.				Bac. typhosus					Viv. cholerae				
	Acids	PH \ time	h 6	9	12	24	m 90	h 2	3	6	m 90	h 2	3	6	9	m 5	10	15	20	30
C_3	Propionic	3.4	+	+	−	−	+	+	−	−	+	+	±	−	−	+	+	+	−	−
	Acrylic	3.1	+	−	−	−	+	−	−	−	+	±	−	−	−	+	±	−	−	−

C_4	Butyric	3.4	+	+	−	−	+	+	−	−	+	+	±	−	−	+	+	+	−
	Isobutyric	3.4	+	+	+	−	+	+	+	−	+	+	+	±	−	+	+	+	+
	Crotonic	3.3	+	±	−	−	+	±	−	−	+	+	−	−	−	+	+	+	−
Control			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(II)—Results at $N/1000$ and saturated aqueous solution at 20°C.

Number of C atom	Microorganisms: Acids	PH \ time	Staph. c' pyogen. aur m 60	90	h 12	24	36	d 2	5	6	9	Prot. vulgar. Hauser m 30	45	h 9	12	24	36	d 3	5	7
C_3	Propionic	3.9	+	+	+	+	±	−	−	−	−	+	+	+	+	−	−	−	−	−
	Acrylic	3.6	+	+	+	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−
C_4	Butyric	3.9	+	+	+	+	±	−	−	−	−	+	+	+	+	−	−	−	−	−
	Isobutyric	3.9	+	+	+	+	+	+	−	−	−	+	+	+	+	±	−	−	−	−
	Crotonic	3.8	+	+	+	+	−	−	−	−	−	+	+	+	±	−	−	−	−	−
C_{11}	Undecylic	6.0	+	+	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	−
	Undecylenic	5 4	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
C_{18}	Stearic	6.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Oleic	6.0	+	+	+	+	+	+	±	−	−	+	+	+	+	+	+	+	−	−
Control			+	+	+	+	+	+	[illegible]	+	−	+	+	+	+	+	+	+	±	−

Number of C atom	Microorganisms: Acids	PH \ time	Bac. typhosus m 45	60	h 12	24	36	d 3	4	5	9	Vib. cholerae m 1	2.5	5	45	60	90	h 2	3	6
C_3	Propionic	3.9	+	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	Acrylic	3.6	+	+	±	−	−	−	−	−	−	+	+	+	±	−	−	−	−	−
C_4	Butyric	3 9	+	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	Isobutyric	3.9	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	−	−	−
	Crotonic	3.8	+	+	+	±	−	−	−	−	−	+	+	+	+	+	−	−	−	−
C_{11}	Undecylic	6.0	+	+	+	+	−	−	−	−	−	+	±	−	−	−	−	−	−	−
	Undecylenic	5.4	+	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−
C_{18}	Stearic	6.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	Oleic	6.0	+	+	+	+	+	+	+	−	−	+	+	+	+	+	±	−	−	−
Control			+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+

From the results noted in Table 5 (I), (II), we ascertained the following facts. If we compare the sterilizing power at the same molecular concentration, we find that unsaturated monobacic fatty acids have stronger sterilizing action than saturated monobasic fatty acids.

We find also in saturated aqueous solution at 20°C, unsaturated monobasic fatty acids have stronger sterilizing action than saturated monobasic fatty acids. This phenomena is especially distinctly by C_{11} acids.

(6) Sterilizing action at the same pH of monobasic fatty acids.

To find the sterilizing action at the same pH, and to compare the sterilizing action at the same molecular concentration of monobasic fatty acids, I made solutions of pH 3.0 and pH 4.0 with each acid, and compared them at the same number of C atom. Acids of C_{11} and C_{18} were compared at pH 6.0 and pH 6.2 respectively. Results are as shown in Table 6. (I), (II).

To make the solution of pH 6.0 with saturated undecylenic acid solution of pH 5.4, we must dilute the acid with distilled water 20 times by volume. Also to make the solution of pH 6.2 with oleic acid of pH 6.0, we must dilute the acid with distilled water 5 times by volume.

Table 6.—Sterilizing action at the same pH of monobasic fatty acids.

(I)—pH 3.0

Number of C atom	Microorgnism		Staph c. pyog				Prot vulgar. H				Bac. typhosus.				Vib. cholerae			
	Acids	time / PH	h 2	3	6	9	m 45	60	90	h 2	m 60	90	h 2	3	m 1	2 5	5	10
C_3	Propionic	N/20	+	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−
	Acrylic	N/80	+	+	+	−	+	+	±	−	+	+	±	−	+	+	+	−
C_4	Butyric	N/20	+	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−
	Isobutyric	〃	+	+	−	−	+	+	−	−	+	+	+	−	+	+	−	−
	Crotonic	N/40	+	+	±	−	+	+	±	−	+	+	+	−	+	+	±	−
Control			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(II)—pH 4.0, pH 6.0, pH 6.2

Number of C atom	Microorganism		Staph. c. pyogen.								Prot. vulgar. H.							
	Acids	time / conc.	h 9	12	36	d 2	3	4	5	8	h 2	3	12	24	36	d 2	5	6
C_3	Propionic	N/2000	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−
	Acrylic	N/8000	+	+	+	+	+	−	−	−	+	+	+	+	+	−	−	−
C_4	Butyric	N/2000	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−
	Isobutyric	〃	+	+	+	+	−	−	−	−	+	+	+	±	−	−	−	−
	Crotonic	N/4000	+	+	+	+	±	−	−	−	+	+	+	+	±	−	−	−
C_{11}	Undecylic	satur.	+	+	+	+	−	−	−	−	+	+	+	−	−	−	−	−
	Undecylenic	〃	±	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−

C_{18}	**Stearic**	satur.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	Oleic	〃	+	+	+	+	+	+	±	−	+	+	+	+	+	+	−	−
	Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	−

Number of C atom	Microorganism		Bac typhosus.								Vib. cholerae							
	Acids	conc \ time	h 6	12	24	36	d 2	3	4	7	m 5	10	30	60	90	h 2	3	3
C_3	Propionic	N/2000	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−
	Acrylic	N/8000	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−	−
C_4	Butylic	N/2000	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	−
	Isobutyric	〃	+	+	+	+	−	−	−	−	+	+	+	+	+	−	−	−
	Crotonic	N/4000	+	+	+	+	±	−	−	−	+	+	+	+	+	−	−	−
C_{11}	Undecylic	satur	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−
	Undecylenic	〃	±	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−
C_{18}	Stearic	〃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±
	Oleic	〃	+	+	+	+	+	+	−	−	+	+	+	+	±	−	−	−
	Control		+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+

From the results noted in Table 6. (I), (II), we find the following facts. If we compare the sterilizing power of C_3 and C_4 at the same pH, we find that unsaturated acids have weaker sterilizing power than saturated acids. And then anions of these acids have no sterilizing power. (Table 4. (I), (II).) Accordingly, the molecular concentration of these acids has a great effect on sterilization. Concerning the sterilization of acids of C_{11} and C_{18}, unsaturated monobasic fatty acids have stronger sterilizing power than saturated acids, even if unsaturated acids, are adjusted to the same pH. And these anions have sterilizing power respectively. But if we compare the results noted in Table 3 and Table 6, we find that undissociated molecules of these acids have considerable sterilizing power.

Summary.

I studied the sterilizing action of unsaturated monobasic fatty acids on putrifactive bacteria, Bac. typhosus and Vib. cholerae. Results are as follows.

(1) At the same molecular concentration, acrylic acid (C_3) and crotonic acid (C_4) have nearly the same sterilizing power. But in detail, acrylic acid has the slightly stronger sterilizing power than crotonic acid. Undecylenic acid (C_{11}) is very slightly soluble in water, but its sterilizing action is very strong.

(2) Anions of C_3 and C_4 acids have no sterilizing action.

(3) Anions of C_{11} acid has strong sterilizing action.

(4) At the same molecular concentration, unsaturated monobasic fatty acids have stronger sterilizing power than saturated manobasic fatty acids. This fact is distinct by C_{11} acid.

(5) At the same pH of acids of C_3 and C_4, saturated monobasic fatty acids have stronger sterilizing power than unsaturated acids.

(6) Molecular concentration and undissociated molecules of these acids have a considerable sterilizing power.

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Biochemical Studies on "Miso", Fermented Soy-bean Paste. Part I.

Nutritive Value of "Miso"-Proteins as a Supplement of Rice.

By

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Introduction.

For more than a thousand years "miso" has been used as one of the most important food articles in Japan. Boiled rice and "miso"-soup form an indispensable daily ration for the Japanese people. "Miso" is also largely used with vegetables, fish and meats. Its annual production in recent years is estimated to be worth more than twenty millions of yen, nevertheless the role it plays in nutrition is yet little understood, so that more thorough study of this subject is desirable.

O. Kellner[1] was the first who analysed various kinds of "miso" and pointed out that it was rich both in true albuminoids and soluble carbohydrates and that it was easily digestible and nutritious. Later T. Takahashi[2] investigated the decomposition products of proteins in "Hatchōmiso" (brown miso) and isolated alanine, leucine, proline, glutaminic acid and lysine; besides detecting the presence of aspartic acid, histidine, arginine, tryptophane and cystine.

T. Hara and R. Takata[3] have recently carried out feeding experiments with white rats and observed that the rats fed on a diet in which "miso" had been added as the sole source of proteins, failed to grow or to sustain life for a long period. They have attributed this to the lack of certain indispensable amino acids, such as cystine and tryptophane in "miso", which might have been partly destroyed during the fermentation process. We see, however, from the chemical analysis that nearly half of the nitrogen contained in "miso" still exists in the form of proteins, even after a long period of fermentation. Recent investigation has further proved that the amino-acids and bases formed by the decomposition of proteins can be again utilized for the formation of body proteins, so it is very probable that these nitrogen compounds contained in "miso" still play an important role in nutrition. Thus we can expect a still better result than that mentioned above, when "miso" is used, not as the sole source of proteins in the diet, but as the supplement of rice proteins.

From such a point of view, the present author has carried out feeding experiments with various kinds of "miso" to determine their relative nutritive value, especially when used as the supplement of rice.

The details are given as follows :——

Experimental.

I. *Experiment with "Sendaimiso".*

The sample used in this experiment was supplied from a "miso" factory in Tōkyō. This kind of "miso" may be taken as standard because it is most widely used and seems to be especially favoured in Tōkyō and in the north-eastern provinces.

It was prepared from 100 parts by volume, of soy-beans, 50 parts rice "koji" (steamed polished rice moulded by a special fungus, Aspergillus oryzae) and 50 parts common salt. The soy-beans were first soaked in water for several hours and steamed (at one pound pressure) for about ten hours until they turned brown. After cooling, they were finely ground, mixed with rice "koji" and salt put in a fermenting tank, covered with a wooden lid, pressed and kept in a cold place. Owing to the presence of a large quantity of common salt and the prevention of the free access of air, the fermentation proceeds very slowly and is only completed after 12 to 18 months.

Recently Y. Sakurai[4] investigated in our laboratory the changes in chemical composition during the ripening of "Sendaimiso" and obtained the following results :

Table I. Changes of the Chemical Composition of "Sendaimiso" during Fermentation. (%)

Composition	Before fermentation	Time of fermentation (Months)				
		1	3	6	9	12
Water	48.52	51.25	50.64	50.20	50.90	52.92
Total-N	1.98	2.10	2.08	2.19	2.08	2.14
Protein-N	1.44	1.28	1.07	1.08	1.03	0.98
N-insol. in water	1.26	1.17	0.99	1.02	0.97	0.94
N-sol. in water	0.72	0.93	1.09	1.17	1.11	1.20
Amino-N	0 29	0.42	0.52	0.51	0.50	0,49
Basic-N	0.24	0.27	0.29	0.31	0.32	0.30
NH_3-N	0.041	0 075	0.100	0.110	0.095	0.110
Reducing sugar	8.95	9.94*	9.36	9 60	9 63	9.49
Starch & dextrin	4 92	3.00	——	——	2 41	2.40
Free acid (as lactic)	1.48	1.39	1.93	1.81	1.93	2.32

From the above table it can be seen that the proteins are gradually dissolved and decomposed until after 12 months they are reduced to 2/3 of the original sample. On the contrary there is a considerable increase of water-soluble nitrogen, including amino, basic and ammoniacal nitrogen. As the proteins in the material are denaturated by heat and fermentation and a part of the amino acids and bases is further decomposed into lower compounds which finally give rise to ammonia, it is natural to expect that the nutritive value is more or less decreased, though it may in some degree be compensated with the increase of digestibility. The chief object of the present investigation is to determine the effect of proteins in various kinds of "miso" upon the growth of rats when used as the supplement of rice, and at the same time to compare them with the proteins of "kōridōfu" (frozen soy-bean-curd) and fish meat powder.

Experiment (A).

Table II. Diets used in the Experiment. (g)

Composition of diets	(I) Control group	(II) Miso group	(III) Kōridōfu grop	(IV) Fish powder group
Polished rice	94.0	79.0	85.8	88.2
Sendaimiso	——	20.0*	——	——
Kōridōfu	——	——	9.2	——
Fish meat powder	——	——	——	5.8
Ash of miso	5.0	——	5 0	5.0
$CaCO_3$	1.0	1.0	1.0	1.0
Butter**	7.5	7.5	5.0	7.5
Oryzanin***(c.c.)	5.0	5.0	5.0	5 0

As dry matter
Preparation of the Meiji Confectionary Company.
Preparation of the Sankyō Pharmaceutical Co.

The protein contents and the calories of the above diets were as follows:

Table III. Protein Contents and Calories of the Diets. (%)

	(I) Control group	(II) Miso group	(III) Kōridōfu group	(IV) Fish powder group
Proteins in polished rice	6.1	5.1	5.5	5.7
Proteins in miso, kōridōfu and fish powder	—	5.4	5.3	5,5
Total protein	6,1	10.5	10.8	11.2
Total-N	0.98	1.67	1.72	1.79
Calories	328.5	345,7	349.3	358.0

As is shown in the above table III the diet of the control group (I) contained only 6.1% protein, while in those of (II), (III) and (IV) groups the protein contents were increased to 10.5, 10.8 and 11.2% respectively by the addition of "miso", "kōridōfu" and fish meat powder. The calorific values were nearly the same, except for those in the control group.

Experiment (B).

The composition of diets in this experiment were essentially the same as those of the previous experiment (A) except that the polished rice which contains no embryo was replaced by that containing the embryo ("haigamai"). For simplicity the latter will be denoted as "polished embryo rice".

Table IV. Protein Contents and Calories of the Diets. (%)

	(I) Control group	(II) Miso group	(III) Kōridōfu group	(IV) Fish powder group
Proteins in polished embryo-rice	6.9	5.8	6.3	6.5
Proteins in miso, kōridōfu and fish powder	—	5.4	5.3	5.5
Total proteins	6.9	11.2	11.6	12.0
Total-N	1.10	1.78	1.84	1.93
Calories	334.5	351.8	354.9	364.3

Young albino rats weighing about 30～40 g. were previously fed for one week with unpolished rice flour, and those which had grown at a normal rate were taken and fed on the above diet. The ingredients were thoroughly mixed with water and cooked in a water bath for about half an hour. During the first 20 days each group consisting of two rats was given daily 15 grams of the diet and after that time 20 grams. The experiment lasted for 68 days from October to December. The growth curves are shown in the following charts:

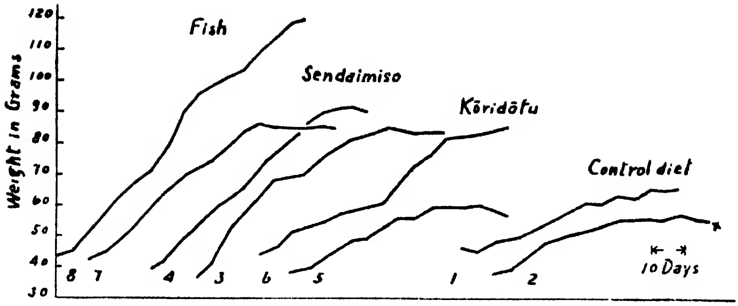


Chart I. Experiment (A). Growth curves of male rats fed on polished rice supplemented with "Sendaimiso", "kōridōfu" and fish powder.

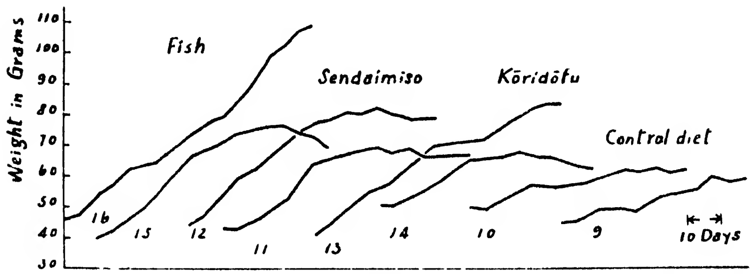


Chart II. Experiment (B). The same fed on polished embryo-rice supplemented with "miso", "kōridōfu" and fish powder.

From the above charts we see that the growth curves are in order of fish powder (IV), "miso" (II), "kōridōfu" (III) and control group (I). The last group attained 60 grams.

II. Experiment with various kinds of "Miso".

The samples used in this experiment were "Shiromiso", "Yedomiso", "Sendaimiso", "Inakamiso" and "Hatchōmiso", "kōridōfu" and fish meat powder were also used for comparison. By the preparation of these kinds of "miso", the raw materials were mixed in the following proportion :

Table V. Raw Materials of "Miso". (By Volume)

Kind of Miso	Soy-bean	Polished rice	Barley	Salt
Shiromiso	1.00	2.00	——	0.30
Yedomiso	1.00	1.00	——	0.40
Sendaimiso	1.00	0.30	——	0.40
Inakamiso	1.00	——	1.00	0.46
Hatchōmiso	1.00	——	——	0.25

The soy-beans were steeped and steamed for several hours according to the kind of "miso" used and while still hot they were thoroughly mixed with

rice-"koji" and common salt. For "Inakamiso" barley "kōji" was used instead of rice-"kōji", and for "Hatchōmiso" soy-bean "kōji" alone was used. The mixture was then pressed into a fermenting tank. In "Shiromiso" the ripening was completed in 3～5 days, in "Yedomiso" in 10～30 days while in "Inakamiso" and in "Sendaimiso" 11～12 months were required for complete ripening. The chemical composition of these samples were as follows :

Table VI. Chemical Composition of the Samples used in the Experiment. (%)

Material	Water	Crude protein	Pure protein	Carbohydrates	Crude fat	Ash	NaCl
Shiromiso	52.00	9.31	6.44	31.90	2.70	6.12	4.71
Yedomiso	46.48	12.63	7.31	23.90	5.32	8.25	6.35
Sendaimiso	52.92	13.38	6.13	11.89	3.95	13.96	11.36
Inakamiso	54.60	12.00	5.19	8.61	5.21	13.75	11.71
Hatchōmiso	46.37	21.00	10.94	4.89	6.15	12.88	9.78
Polished rice	7.68	7.46	7.36	79.98	0.81	0.65	——
Polished embryo-rice	7.20	8.35	8.19	78 63	1.30	0.78	——
Kōridōfu	6.45	58.27	58.15	5.20	26 88	4.55	——
Fish powder	2.48	97.81	97.36	——	——	1.13	——
Soy-beans	6.99	35.50	35.28	26.33	19.52	5.01	——
Patrogen(5)	1.24	14.63	——	68.31	10.94	4.68	——

These samples were mixed in the following proportion :

Table VII. Compsition of the Diets.

Material	(I) Shiromiso group	(II) Yedomiso group	(III) Inakamiso group	(IV) Hatchōmiso group
Polished rice	68.7	74.4	74.0	82.7
Shiromiso	27.6	——	——	——
Yedomiso	——	22.7*	——	——
Inakamiso	——	——	24.8*	——
Hatchōmiso	——	——	——	13.7*
Ash of Sendaimiso	1.8	1.7	- -	1.7
Soy-bean oil	0.9	0.2	0.2	0.9
$CaCO_3$	1.0	1.0	1.0	1.0
Butter**	7.5	7.5	7.5	7.5
Oryzanin (c.c.) ***	5.0	5.0	5.0	5.0

Material	(V) Control group	(VI) Sendaimiso group	(VII) Kōridōfu group	(VIII) Fish powder group
Polished rice	91.5	79.3	84.8	86.0
Sendaimiso	— —	18.8*	——	——
Kōridōfu	——	——	9.2	——
Fish powder	— —	——	——	5.5
Ash of Sendaimiso	5.0	——	5.0	5.0
Soy-bean oil	2 5	0.9	——	2.5
$CaCO_3$	1 0	1.0	1.0	1.0
Butter**	7.5	7.5	7.5	7.5
Oryzanin (c.c.)***	5.0	5.0	5.0	5.0

* As dry matter.
** Preparation of the Meiji Confectionary Co.
*** Preparation of the Sankyō Pharmaceutical Co.

Experimental animals.

Sixty five spring-born rats weighing 30~40 g. (average 35.1 g.) were picked out and fed on "Patrogen"* (a kind of milk powder containing all the dietary factors essential for the growth of children). The average body weight increased from 35.1 to 52.2 g. in 10 days. The growth rates being nearly the same as those of Donaldson, Dunn and Watson[6], as is shown in the charts :

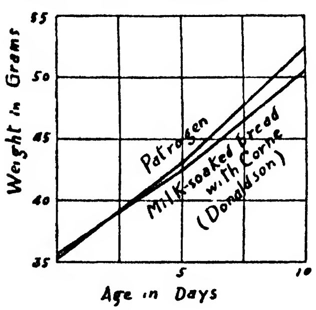


Chart III. Growth curves during preliminary feeding.

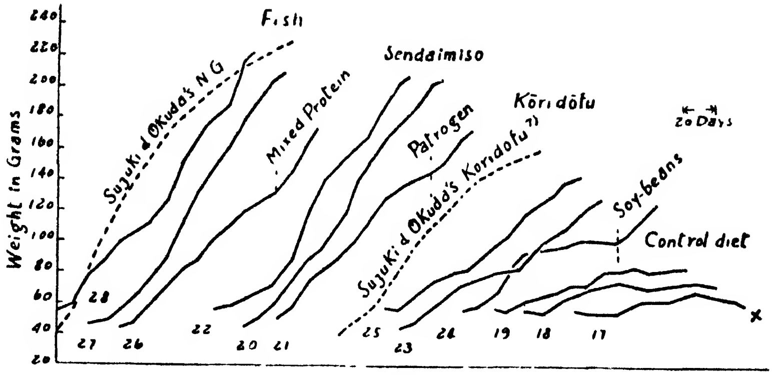
From 65 rats reared as above stated, 36 healthy ones were selected and divided into three groups, each consisting of three rats which were fed on the diets shown in Table VII.

Experiment (A).

The growth curves are shown in chart IV and V.

Fig. I shows that the rats fed on "Sendaimiso" grew healthy but those (Fig. II) fed on the "Hatchōmiso" diet were still better as shown in Table VII. These fed on the "Inakamiso" diet (Fig. III) grew more slowly at first, but continued to grow until the end of the experiment, while the rats fed on the "Shiromiso" diet exhibited a remarkable growth for some time, but during the last forty days no noticeable increase in weight was observed. The animals (Fig. IV) fed on the fish diet grew at an approximately normal rate, while those fed on the "kōridōfu" diet hardly reached 108 or 130 g. in 100 days. This is in accordance with the observation of Suzuki, Okuda and

* Preparation of the Meiji Confectionary Company.



× ……indicates death.

Chart IV. Showing growth curves of male rats fed on the polished rice diet supplemented with "Sendaimiso", "kōridōfu" and fish meat powder respectively.

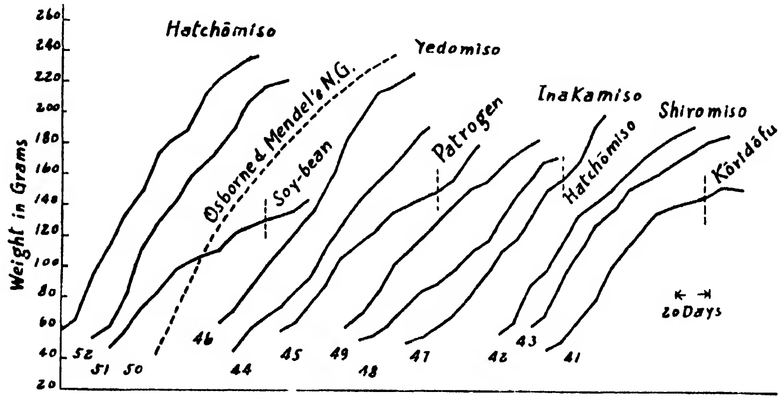


Chart V. Showing growth curves of male rats fed on the polished rice diet supplemented with various kinds of "miso".

Matsuyama(7) who attributed the lower nutritive value of "kōridōfu" to the loss of certain proteins of high nutritive value during the process of making.

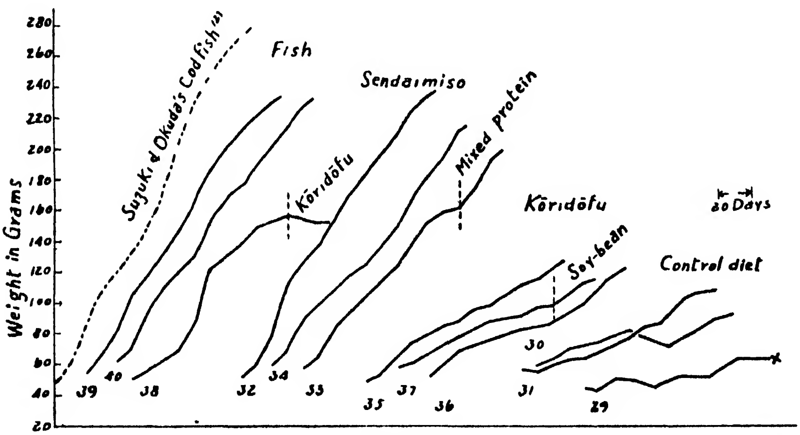
From these results, it can be seen that the greater the amount of soybeans in "miso", the higher is the supplementary value. The fact is in accordance with the observation of Daniels and Nichols(8), Osborne and Mendel(9), McCollum, Simmonds and Parsons(10), and Johns and Finks(11), namely that soybean proteins are capable of supplementing the low nutritive value of cereals.

Experiment (B).

Polished embryo-rice supplemented by "Sendaimiso", "kōridōfu" or fish, produced better growth results than the polished rice diet. The rats (R. 39 and R. 40) fed on the fish diet showed the best development and almost the same appearance as that of rat(12) No. (2), with a diet consisting of 10～18% codfish protein, 43～52% starch, 27～28% protein free milk and 10～

18% butter. Those fed on the "Sendaimiso" diet continued to grow at an approximately normal rate, while those fed on the "kōridōfu" diet gained in weight more slowly. Animals on the control diet containing 7.7% protein of polished embryo-rice have shown very slow growth, one of them gradually declined in weight and finally died, as did the rats on the control diet with polished rice. When rats Nos. 24 and 37 previously fed on the "kōridōfu" diet, were changed to the soy-bean diet at the same level of protein intake, they became more healthy in every respect than the rest of the group. The rats No. 33 on "Sendaimiso" diet, and No. 26 on fish, when placed on a 20% protein intake, 65.5% polished rice, 18.8% "Sendaimiso", 9.2% "kōridōfu" and 5.5% fish, showed an accelerated growth response. The rats No. 21 on the "Sendaimiso" diet and No. 48 on the "Yedomiso" diet, also showed the remarkably good quality of the protein of "Patrogen".

The growth curves are shown in the following chart.



× ··· · indicates death.

Chart VI. Showing growth curves of male rats fed on the polished embryo-rice diet supplemented with "Sendaimiso", "kōridōfu" and fish powder respectively.

The average gain of each group and the food intake in 100 days are as follows :

Table VIII. Rate of Growth. Rats on the polished rice diet.

Kind of diet	No. of rats	Durations of ex- periments	Initial body weigt grams	Gain in 125 days grams	Gain in 100 days grams	Average gain in 100 days grams	Food intake per rat in 100 days grams*
Control diet	17	118	57	——	8	15.7	500
	18	150	58	73	17		
	19	150	58	84	22		

* The daily food intake was only approximately estimated.

Sendamiso diet	20 21 22	150 100 150	46 53 57	218 —— 212	130 91 115	112.0	1140
Kōridōfu diet	23 24 25	150 100 150	44 58 59	130 —— 144	54 44 66	54 7	860
Fish powder diet	26 27 28	100 150 150	42 45.5 55	—— 211 223	92 131.5 126	116 5	1080
Shiromiso diet	41 42 43	100 150 150	46.5 56 59	—— 193 187	99 5 127.0 115	113 5	1190
Yedomiso diet	44 45 46	150 100 150	42 5 56 59	193 —— 227	120.5 91 150	120 5	1160
Inakamiso diet	47 48 49	100 150 150	49 50 59	—— 173 185	103 90 104	99.0	1060
Hatchōmiso diet	50 51 52	100 150 150	46 51 57	—— 222 236	83 150 156	129.7	1250

Rats on the polished-embryo rice diet.

Control diet	29 30 31	119 150 150	42 56 56	—— 92 107	19 20 37	25.3	660
Sendaimiso diet	32 33 34	150 100 150	49 54 58	237 —— 215	157 106 123	129.3	1200
Kōridōfu diet	35 36 37	150 150 100	46 50 56	127 123 ——	63 48 42	51.0	850
Fish powder diet	38 39 40	100 150 150	48 51 56	—— 234 233	109 156 142	135.7	1160

Experiment (C).

In the preceeding experiments (A) and (B), the daily ration during the first 40 days was 10 g. per rat, theieafter it was increased to 15 g. After

125 days when the experiment was finished, most of the animals were used for another experiment. The composition of each diet was the same as before, but the quantity was so adjusted that each rat received exactly 0.2 g. nitrogen per day. This corresponds to 11~12 g. diet according to the kind of "miso" to be tested. After the preceeding investigations, the following 50 days, i.e. from the 126~175 th day (171 st~195th day from the birth), were divided into two periods. During the first period (126~150th day) each rat received the same diet as in previous feeding and in the second period (151st ~175th day) the diet was changed, and the average rate of growth in both periods was estimated, for instance:

1. $$\frac{\text{Aver. rate of growth in the second period on "kōridōfu" diet}}{\text{" " " in the first period on "Hatchōmiso" diet}} = 0.6.$$

2. $$\frac{\text{Aver. rate of growth in the second period on "Sendaimiso" diet}}{\text{" " " in the first period on "Yedomiso" diet}} = 0.9.$$

3. $$\frac{\text{Aver. rate of growth in the second period on "Sendaimiso" diet}}{\text{" " " in the first period on "Inakamiso" diet}} = 1.2.$$

4. $$\frac{\text{Aver. rate of growth in the second period on "Hatchōmiso" diet}}{\text{" " " in the first period on "Shiromiso" diet}} = 1.5.$$

5. $$\frac{\text{Aver. rate of growth in the second period on "Yedomiso" diet}}{\text{" " " in the first period on "fish powder diet}} = 0.8.$$

6. $$\frac{\text{Aver. rate of growth in the second period on "Patrogen" diet}}{\text{" " " in the first period on "Sendaimiso" diet}} = 0.9.$$

From Donaldson, Dunn and Watson's data, relating to the growth of male rats of corresponding age fed on the adequate diet (milk soaked bread and corn), the following was calculated.

$$\frac{\text{Aver. rate of growth during 25 days from 196~220th day (from the birth)}}{\text{Aver. rate of growth during 25 days from 171~195th day (from the birth)}} \cdot$$

$$= \frac{0.43}{0.53} = 0.8$$

In the present experiment, when one and the same diet was given during both periods, the ratio was found to be about 0.8. Therefore the author assumed that, when the diet in the second period is changed and the ratio becomes larger than 0.8 it indicates that the nutritive value of the second diet is higher than that of the first, and vice versa.

From the experimental data given above, we see that "kōridōfu" and "Shiromiso" are lower in value than "Hatchōmiso". "Inakamiso" is lower

than "Sendaimiso" and the latter is equal to or a little better than "Yedomiso" but inferior to "Patrogen", etc.

These results nearly agree with those of the experiments (A) and (B). It should be mentioned here that, the amount of non-protein-N in "miso" is always higher than in "kōridōfu" or in fish powder, nevertheless, the rate of growth, induced by the same quantity of N., i.e. by 0.2 g. N. per day, is higher in "miso" than in "kōridōfu", though a little lower than in fish powder. Thus it may be assumed that the non-protein-N in "miso" is utilized, at least partly, for building up the body protein in rats.

This confirms the observations of S. Maeda[11], that the acid hydrolytic products of proteins when supplemented with tryptophane, can fully replace the proteins in a given diet.

The following analytical data will serve to give some idea of the relation between the constituents and the nutritive value of proteins in various kinds of food articles, though there exists some unknown factor which plays an important role in the nutritive value of proteins.

Table IX. Distribution of Nitrogen in the Food Materials used in the Preceeding Experiments. (%)

	Water	Total-N	Arginine-N	Histidine-N	Lysine-N	Cystine-N	Tryptophane
Polished rice[14]	7.68	1.19	0.15	0.10	0.06	0.01	0.09
Polished embryo-rice	7.20	1.34	0.24	0.09	0.06	0.01	0.10
Siromiso	52.00	1.49	0.15	0.05	0.09	– –	0.11
Yedomiso	49.48	2.02	0.13	0.05	0.08	- —	0.12
Sendaimiso	52.92	2.14	0.17	0.06	0.07	- -	0.08
Inakamiso	54.60	1.92	0.13	0.05	0.07	- -	0.07
Hatchōmiso	46.37	3.36	0.18	0.09	0.14	----	0.05
Kōridōfu	6.45	9.30	1.18	0.30	0.57	0.08	0.85
Fish powder	2.48	15.65	1.93	0.52	1.72	0.11	1.18
Soy-bean [15]	6.99	5.68	0.88	0.15	0.40	0.09	0.51
Rat muscle	2.10	16.21	1.98	0.55	1.76	0.14	1.26

Arginine, histidine and lysine were estimated by Van Slyke's method. Cystine-N by Okuda's method[16] and tryptophane by May & Rose's method improved by Matsuyama & Mori[17].

Summary.

(1) A series of feeding experiments was carried out with white rats to determine the nutritive value of various kinds of "miso", when used as a supplement of polished rice.

(2) The supplementary value of "miso" proteins was found to lie between fish proteins and "kōridōfu", in the order of "Hatchōmiso", "Sendaimiso",

"Yedomiso", "Shiromiso" and "Inakamiso", the last being the lowest.

(3) The nutritive value of "miso" seems to depend chiefly on the amount of soy-beans, used as raw material, and not on the duration of fermentation. The lower value of "Inakamiso" may be explained by the lower content of soybeans.

(5) These experiments have clearly shown that "miso" plays a very important role in the nutrition of the Japanese people, especially in supplying the deficiency of rice proteins.

My best thanks are to Professors U. Suzuki and R. Sasaki for their helpful criticism and encouragement throughout these investigations. I am also greatly indebted to Mr. Sakurai who kindly consented to carry out the chemical analyses and to the Trade Association of the Tōkyō Miso Manufacturers, for kindly supplying the funds and the specimens of soy-bean pastes used in these experiments.

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Fig I Rat No 20, fed on "Sendaimiso" 176 g after 100 days.

Fig. II Rat No 52, fed on "Hatchomiso" 213 g. after 100 days.

Fig. III Rat No 49, fed on "Inakamiso" 163 g after 100 days.

Fig IV Rat No 28, fed on fish 181 g. after 100 days.

Fig V Rat No. 25, fed on "kōridōfu" 125 g after 100 days.

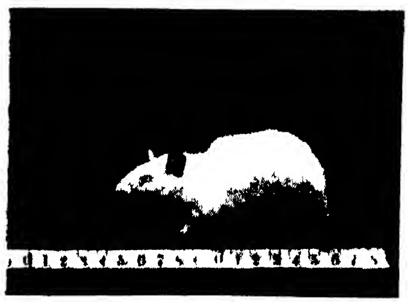

Fig. VI Control rat No. 19, fed on polished rice diet, 80 g. after 100 days.

Investigation on the Influence of Aerial-Earth Circuit on the Biological Activities.

II. Mechanism of the influence on Azotobacter chroococcum as to its potential.

By

Arao ITANO. Ph. D.

(Received August 13, 1933).

As the first report[1] in this series of investigation, it was experimentally proven that the growth as well as the fixation of atmospheric nitrogen of Azotobacter chroococcum was activated in the closed aerial-earth circuit and even by earthing only. Subsequently an enquiry was made as to the mechanism of activation in regard to the potential of Azotobacter chroococcum culture, and the results are reported here as the second report.

Experimental Procedure.

The same strain of Azotobacter chroococcum and the medium were used as noted in the first report, but the flasks were equipped slightly differently so that the potential of the culture is determined easily and all the manipulations can be carried out aseptically, as shown in Fig. I.

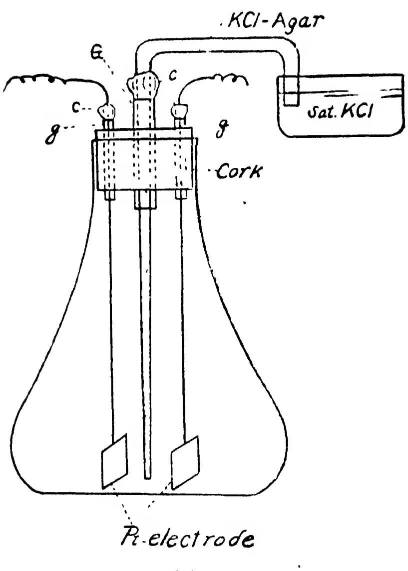

Fig. I.

Each flask was provided with a cork which has two glass tubes, g (4 mm. diameter) through either one or both, the platinum electrode is placed and held in position, and plugged with cotton. An another glass tube, G (8 mm. diameter) with cotton plug c was provided through which a sample for pH determination is taken and also an agar bridge is inserted when the potential is determined. The agar bridge is drawn to capillary at its end and considerably longer than the depth of the flask so that the tip can be broken off at every determination to keep it from contamination. The outer surface of the bridge is sterilized with alcohol and flamed before used. All through

the investigation, very careful precautions were taken against contamination and agitation.

The potential of Azotobacter chroococcum culture was determined against the sterile culture medium and also against the saturated calomel electrode by the following chains I and II respectively, using the K-type potentiometer:

Pt (blank)	Sterile culture medium.	Sat. KCl (agar-bridge.)	Culture	Pt (blank)·· ········ (I)
Hg-HgCl	Sat. KCl	Sat. KCl (agar-bridge.)	Culture	Pt (blank)···· ··· ···(II)

The electromotive force thus determined, was converted into Eh, the standard E. M. F., or the normal hydrogen electrode potential. At the same time, the hydrogen electrode potential was determined experimentally, and the difference between that and Eh is given so that the comparable intensity can be inferred.

Influence of Aerial-Earth Circuit on the Sterile Medium.

First it was attempted to ascertain if the potential of the sterile medium is influenced to any extent by the aerial-earth circuit, as follows:

Four Erlenmyer flasks with 100 c.c. sterile Ashby's solution were taken and treated in the following manner: Fask I connected to the antenna and

Table I.—*Influence of Aerial-Earth Circuit on the Sterile Medium.*

Flasks*	Time in hours.	0 (volts)	24 (volts)	48 (volts)	72 (volts)	96 (volts)	120 (volts)
I.	Potential determined	0.8205	0.1454	0.1446	0.1558	0.1574	0.1566
	H_2-electrode potential	0.9355	0.9360	0 9390	0.9440	0.9430	0.9410
	Eh**	0.3675	0.3924	0.3916	0.4028	0.4044	0.4036
	Difference	0.5680	0.5436	0.5474	0.5412	0.5386	0.5374
II.	Potential determined	0.1257	0.1369	0.1383	0.1261	0.1439	0.1250
	H_2-electrode potential	0.9315	0.9400	0.9440	0.9385	0.9400	0.9430
	Eh	0.3737	0.3839	0.3853	0 3731	0.3909	0.3720
	Difference	0 5578	0.5561	0.5587	0.5654	0.5491	0.5710
III.	Potential determined	0.1205	0.1478	0.1556	0.1653	0.1681	0.1589
	H_2-electrode potential	0.9315	0.9410	0.9400	0.9440	0.9385	0 9440
	Eh	0.3675	0.3948	0.4026	0.4123	0 4151	0.4059
	Difference	0.5640	0.5432	0.5374	0.5317	0.5234	0.5381
IV.	Potential determined	0.1262	0.1296	0.1262	0.1280	0.1290	0.1287
	H_2-electrode potential	0.9300	0.9410	0.9400	0 9465	0.9385	0.9205
	Eh	0.3732	0.3766	0.8732	0.3750	0.3760	0.3757
	Difference	0.5568	0.5644	0.5668	0.5715	0.5625	0.5448

Notes: *...Flask I. antenna and earthed; II. earthed only; III. antenna only; IV. no outside connection.

**...Eh, the potential of medium converted into the standard, or the normal hydrogen electrode potential.

earthed; Flask II earthed only; Flask III connected to the antenna only; Flask IV no outside connection. The potential was determined at 24 hours intervals under aseptic conditions. The results are given in Table I.

Table I indicates that the potential of Flask IV which is the control, was not changed to any extent, although in the other flasks, the potential increased slightly.

Influence of Aerial-Earth Circuit on the Potential of Azotobacter chroococcum Culture.

The potential of Azotobacter chroococcum culture against the sterile medium and also against the saturated calomel electrode, was determined as follows:

Four flasks A, B, C and D which were treated same as Flask 1, II, III and IV respectively in the pievious case, except all these flasks were inoculated with 1 c.c. of 48 hours old culture of Azotobacter chroococcum. To

Table II.—*Influence of Aerial-Earth Circuit on the Potential of Azotobacter chroococcum Culture.*

Flask*	Time in hours	0 (volts)	24 (volts)	48 (volts)	72 (volts)	96 (volts)	120 (volts)
A.	Potential determined	0.1612	0.0685	0 0906	0.0447	0.0262	0.0453
	H_2 electrode potential	0 9315	0.9220	0.9340	0.9210	0.9205	0.9205
	Eh**	0 4082	0.3155	0 3376	0.2917	0.2732	0.2923
	Difference	0.5233	0 6065	0.5964	0.6293	0.6473	0.6282
B.	Potential determined	0.1717	0.0650	0.0649	0.0331	0.0223	0.0308
	H_2-electode potential	0.9300	0.9140	0.9260	0.9320	0.9320	0.9320
	Eh	0.4187	0.3120	0.3119	0.2801	0.2693	0.2778
	Difference	0.5113	0.6020	0.6141	0.6519	0.6627	0.6542
C.	Potential determined	0.1629	0 0514	0.0411	0 0083	−0.0234	−0.0307
	H_2-electrode potential	0.9335	0.9190	0.9170	0.9140	0.9280	0.9280
	Eh	0.4099	0.2984	0.2881	0.2553	0.2236	0.2163
	Difference	0.5236	0.6206	0.6289	0.6587	0.7044	0.7117
D.	Potential determined	0.1557	0.0496	0.0348	0.0009	−0.0127	−0.0193
	H_2-electrode potential	0.9330	0.9180	0.9150	0.9150	0.9290	0.9280
	Eh	0.4027	0.2966	0.2818	0.2479	0.2343	0.2277
	Difference	0.5303	0.6214	0.6332	0.6671	0 6947	0.7003
D : IV.	Potential determined	0 0100	−0.0610	−0.0882	−0.0937	−0.1160	−0.1588
	Eh	0.2570	0.1860	0.1588	0.1433	0.1310	0.0882

Notes: *...Flasks A, B, C and D were treated same as Flasks I, II, III and IV. in Table I respectively but inoculated.

**...The potential of Flask D determined against Flask IV.

determine the potential of the culture against the sterile medium, Flask D was chained against Flask IV. The results are shown in Table II and Fig. II.

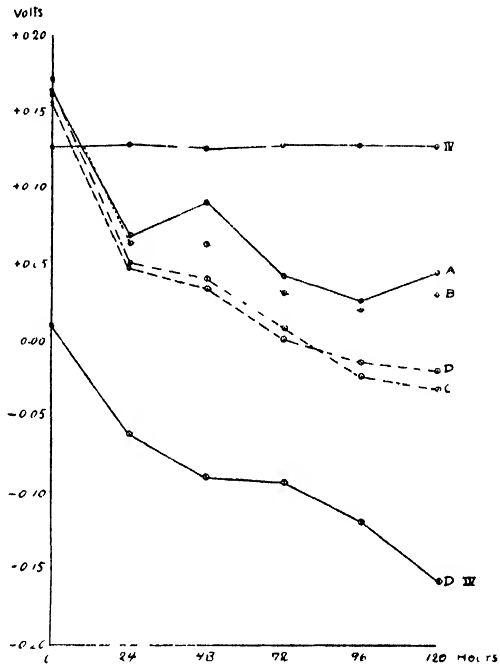

Fig. II—Potential of Azotobacter chroococcum under various treatment.

Notes: A—antenna and earthed ; B—earthed only ; C—antenna only ; D—normal control ; IV—sterile medium.

Table II and Fig. II indicate that the potential difference is plainly determined between the culture and the sterile medium. The potential in all the inoculated flasks showed a sudden drop within the first 24 hours, but after that the drop was different by the flask under different treatment. In flasks I, the least decrease of potential took place which was followed by Flask II while in Flask III and IV, the negative potential was observed.

Discussions.

So far as the the auther is aware, no previous investigation of this nature on Azotobacter chroococcum has ever been undertaken. Consequently it is very difficult to give a satisfactory interpretation of the results obtained.

As to the nature of potential measured in the bacterial culture, Potter(2) who first measured the potential of Bac. coli in different media and also that of yeast, considered that such potential was electrical. However Gillespie(3) later demonstrated that such potential is a special case of the oxidation-reduction potential indicating the intensity of such reaction. Whatever may be the exact nature of such potential, it is certain that the potential is electrically measurable and such the measurement indicates the intensity of ionic activity in the culture.

In this investigation, it was attempted to ascertain, if such an intensity is influenced by the aerial-earth circuit or not, so that the mechanism of the activation obtained in the previous investigation may be understood.

So far as the data obtained and given in Table II and Fig. II, it is plainly indicated that the different arrangement has different influence on the potential of Azotobacter culture.

Summary.

The results obtained in this investigation indicate that the potential of Azotobacter chroococcum culture is influenced by the aerial-earth circuit.

Considering these results in the light of those which were noted in the first report, the difference of potential which is due to the different treatment, may be a factor bringing about the difference in fixation of nitrogen and growth of Azotobacter chroococcum.

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Ueber die chemischen Bestandteile des Tabaks. III. Mitteilung.

Ueber die Farbstoffe der Tabakblüten. (I).

Von

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(Eingegangen am 20, Juli 1933).

Trotz der zahlreichen Untersuchungen über die Bestandteile des Tabaks ist noch wenig über die der Blüten desselben bekannt. In der zweiten Mitteilung[1] hat der Verfasser die Isolierung eines Flavonglucosids, welches er Tabacinin genannt hat, aus den Tabakblattern beschrieben. In der vorliegenden Arbeit möchte er über seine Forschungen über die Farbstoffe der Tabakblüten berichten.

Nach meiner Erfahrung enthalten die Blüten des Tabaks, der früher eine Zierpflanze war, ausser einem Anthocyan noch ein Flavon, um deshalb die phytobiochemische Beziehung zwischen diesen beiden Farbstoffen zu studieren, versuchte der Verfasser die Isolierung dieser Farbstoffe aus den Tabakblüten. Verfasser teilt hier wegen der geringen Ausbeute an Farbstoffen und ferner der Schwierigkeit der Sammlung der Blüten wegen seine noch unvollendeten Untersuchungen nur vorläufig mit.

A. Ueber das Anthocyan.

Die Blüten des Tabaks sind sehr arm an Anthocyan. 63000 von den Kelchen befreite Blüten lieferten 1260 g. trockenes Blütenmehl, woraus nur 0.6 g. Anthocyan-Pikrat isoliert wurden. Die Extraktion des Farbstoffs erforgte mittels methylalkoholischer Salzsäure. Zur Reinigung wurde zuerst das Anthocyan in Bleisalz und dann in Pikrat verwandelt. Der geringe Ausbeute wegen musste der Verfasser auf Krystallisationsversuche verzichten. Die Hydrolysenversuche erwiesen aber dass das isolierte Anthocyan schon genügend rein ist; es besteht aus 1 Mol. Monosaccharid und 1 Mol. Anthocyanidin. In späteren Untersuchungen will ich die Identifizierung des Zuckers und freien Farbstoffs ausführen.

Experimentelles.

(1) Isolierung: — Die frisch gepflückten Tabakblüten wurden von den Kelchen befreit und im Trockenschrank bei ca. 60° getrocknet. 1.2 kg. trocknetes Blütenmehl wurden mit 212 proz. methylakoholischer Salzsäure übergossen, über Nacht stehen gelassen, hierauf abgenutscht und mit 1 proz. methylalkoholischer Salzsäure wiederholt nachgewaschen. Aus dem Extrakt werde der rohe Farbstoff durch Zusatz des dreifachen Volumens Aether gefällt. Diesen Niederschlag löste man in 11 warmem Wasser und fügte dazu die gesättigte Bleiazetatlösung. Der Bleiniederschlag wurde mit Methylalkohol und dann mit Aether gewaschen und hierauf mit 7 proz. methylalkoholischer Salzsäure geschüttelt. Nach der Abtrennung des Bleichlorids wurde das Anthocyan als Chlorid aus dem Filtrat mit Aether niedergeschlagen. Die Fällung nahm man in einer kleinen Menge warmem wasser auf und brachte durch Zusatz von heiss gesättigter Pikrinsäurelösung das Anthocyan-Pikrat zur Ansfallung. Das Pikrat wurde nochmals in 5 proz. methylalkoholischen Salzsäure gelöst und das Farbstoffchlorid erneut durch Aether gefällt. Dieses Chlorid, nach dem Auflösen in warmem Wasser, wurde durch Pikrinsäure wieder in Pikrat verwandelt.

(2) Eigenschaften: — In Wasser oder Methylalkohol löst sich das Anthocyanchlorid mit purpurroter Farbe leicht, aber nicht in Aethyl- oder Amylalkohol. Eisenchlorid ruft in der Lösung des Farbstoffes eine schwach grüne Färbung hervor; Natriumcarbonat gibt eine Violettblau, Bleiazetat eine violettrote Fällung.

(3) Hydrolyse: — 0.2077 g. des bei 100° im Vakuum getrockneten Farbstoff-Pikrats wurden mit 15 ccm 20 proz. Salzsäure 3 minuten gekocht. Nach dem Erkalten wurden das auskrystallisierte, zuckerfreie Farbstoffchlorid abfiltriert und mit etwas 20 proz. Salzsäure nachgewaschen. Die salzsaure Mutterlauge wurde zur Entfernung der gelösten Pikrinsäure ausgeäthert und hierauf das in der Lösung zurückbliebene Anthocyanidin mit Amylalkohol

ausgezogen. Aus den amylalkoholischen Extrakten wurde das Lösungsmittel verdampft, der hinterbliebene Farbstoffrest in Methylalkohol gelöst und mit Aether gefällt.

Die wässerige saure Flüssigkeit, nach Entfernung des gelösten Amylalkohols durch Ausschütteln mittels Aether, wurde mit Natriumbicarbonat neutralisiert, mit Knochenasche entfarbt, filtriert, auf 50 ccm. gebracht und zur polarimetrischen Bestimmung des Zuckers benützt.

In der sauren Spaltung des Pikrats (0.2077 g.) isolierte, bzw. bestimmte ich;

Pikrinsäure······0.0403 g., Anthocyanidin-chlorid····· 0.0965 g.,
Zucker (als Glucose)······0.0429 g.

Eine zweite Hydrolyse führte zu nachfolgenden Werten;

Aus 0.0926 g. des Farbstoff-chlorides.
Anthocyanidin-chlorid··· · 0.0595 g., Zucker (als Glucose) ······0.0228 g.

B. Ueber das Flavon.

Das Tabakblütenmehl, nach Extraktion des Anthocyans, diente zur Isolierung des Flavons. Der Verfasser konnte leider ein reines Flavonglucosid noch nicht herstellen, aber durch die Hydrolyse eines Niederschlags, welcher zweifellos das Flavonglucosid enthalt, eine sehr geringe Menge von dem freien, krystallisierten Flavon erhalten. Ueber den Zucker und das Flavon, die durch die saure Spaltung des rohen Glucosids erhalten wurden, will der Verfasser seine Untersuchungen noch fortsetzen.

Experimentelles.

(1) Isolierung:— Das Blütenmehl des Tabaks, nach Entfernung des Anthocyans mittels methylalkoholischer Salzsaure, wurde mit Calciumcarbonat versetzt und mit 95 proz. Alkohol wiederholt extrahiert. Der alkoholische Auszug wurde mit Natriumhydroxyd neutralisiert, im Vakuum eingedampft, der Rückstand mit warmen Wasser behandelt und filtriert. Aus der wässerigen Lösung erhalt man ein gelbe Fallung durch Zusatz von Bleiazetat. Dieser Niederschlag wurde mit verdünnter Salzsaure zersetzt, abgenutscht und stark eingeengt. Der Rückstand wurde zuerst ausgeathert nnd dann mit Alkohol extrahiert. Die alkoholische Lösung wurde wieder eingeengt und mit Aether gefallt. Ausbeute 1.5 g.

(2) Eigenschaften:— Dieses rohe Glucosid ist hellgelb, und reduziert Fehlingsche Lösung. In der Losung dieses Glucosides gibt Ferrichlorid eine grüne Farbung, Bleiazetat eine gelbe Fällung, Magnesium und Salzsäure ein schönes Rot. Wenn es in konzentrierter Schwefelsäure gelöst wird, so erhält man eine gelbe Lösung mit einer blaugrünen Fluoreszenz.

(3) Hydrolyse:— 1 g. Glucoside wurde mit 100 ccm. 5 proz. schwefelsäure 6 Stunden erhitzt. Die saure Flüssigkeit, nach dem Erkalten, wurde mit Aether geschüttelt und die ätherische Lösung eingedampft. Wird der Rückstand aus verdünntem Alkohol umkrystallisiert, so erhält man gelbe, nadelförmige Krystalle von freiem Flavon. Die schwefelsaure, wässerig Lösung enthält einen Zucker, dessen Identifizierung noch nicht ausgeführt worden ist.

Zum Schluss danke ich herzlich dem Herrn Prof. Y. Okuda für seine Anregung bei dieser Arbeit.

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Sulphates and chlorides as fertilizers (III.)

Viscosity and surface tension of rice paste.

By

Isenosuke ONODERA.

(Received August 3, 1933)

(1) Samples of paddy rice were obtained from culture cylinder in open and paddy fields, the latter of which was devided into small plots by low dykes.

(2) The viscosity of both the polished and unpolished rice increases with the quantites applied, but inverse is true with their surface tension.

(3) The viscosity of the unpolished rice is larger and its surface tension smaller, than those of the polished. This is due to the separating-off of rice bran, containing a large quantity of fat and electrolyte, which have much to do with the viscosity and surface tension.

(4) Relations between the viscosity of the unpolished rice paste and the fertilizer applied were as follows:

(a) The composition of fertilizer, giving the largest viscosity.

Name of experiment		"Uwadai" soil	"Shitadai" soil
Cylinder culture		Ammonium chloride Natrium phosphate Potassium sulphate	Ammonium Sulphate Ca-Superphosphate Potassium Chloride
Field experiment	Mineral fertilizer only	Ammonium Carbonate Ca-Superphosphate Potassium Carbonate	——
	Mineral fertilizer and organic manure	Farm yard manure Soy bean cake Ammonium Chloride Ca-superphosphate Potassium sulphate	——

(b) Relations between viscosity and the compounds of potassium, ammonium so far as their chlorides and sulphates are concerned, were :

The viscosity of the plots of compounds of the same anion are always smaller than those of different anion given, and was no different tendency noticed between the "Uwadai" and the "Shitadai" soils.

(5) Relation between the surface tension of the unpolished rice paste and fertilizer applied were :

(a) The composition of fertilizers, giving the largest surface tension.

Name of experiment		"Uwadai" soil	"Shitadai" soil
Cylinder Culture		Farm yard manure Ammonium chlorides Ca-superphosphate Potassium sulphate	Farm yard manure Ammonium chloride Ca-superphosphate Potassium sulphate
Field experiment	Mineral fertilizer only	Ammonium carbonate Ca-superphosphate Potassium chloride	——
	Mineral fertilizer and organic manure	Farm yard manure Ammonium chloride Ca-superphosphate Potassium chloride Powdered limestone	——

(b) Relations between the surface tension and the compounds of potassium and ammonium, so far as their chlorides and sulphates are concerned, were :

The surface tension of the plots of the compounds of the same anion are generally larger than those of different anion given.

The Chemical composition of tunny liver oil.

By

Tetuo TOMIYAMA

(Received Sept. 21, 1933)

Introduction.

In regard to the fatty acids of aquatic animal oils, much study has been made on their body fats, but comparatively little done on their liver oils. Concerning the study of the body fats of tunny (Thunnus orientalis) there is I. Okada's work, but no researches have been done yet on the fatty acids of its liver oil. Again, with reference to the vitamin-A of tunny liver oil, H. Sekine's work came to our attention years ago, but no determination has yet been made regarding its potency. Lately, Y. Kawakami has estimated by color reaction the quantities of vitamin-A contained in the liver oils of many kinds of fish, and reported that tunny liver oil contained 213.6 units, while cod liver oil, 2.7～7.9 units. The author has made researches on the fatty acids of tunny liver oil and estimated the vitamin-A content by means of biological test. Of course the quantities of these fatty acids vary owing to the factors, such as the seasons of the year, the ages and the sexes of the fish, but it would seem possible to obtain the general inference regarding the contents.

Experiment.

(I) The Preparations of Sample Oil and Mixed Fatty Acids, and Their General Properties.

The oil was obtained by grinding the liver which has passed a day in an ice box after catching the fish in spring time, making it dehydrated powder by mixing with anhydrous Na_2SO_4, then extracting with petroleum ether (b.p.$<50°$). The extract was made to be a constant weight, removing the solvent under the current of CO_2. The oil thus obtained was of liquid form having yellowish brown color, and in winter, it separated a little quantity of solid fat. Its general properties are as shown in Table 1 below. In spring time, the liver contains a large quantity of oil, and the quantity obtained amounted to 26% of the fresh one. As its degree of unsaturation was high and its oxidation during the chemical treatment was considerable, special efforts have been made to perform the following treatments in dried CO_2.

Table 1

Acid value	28.0	Reichert-Meissl value	2.2
Saponification value	177.6	Polenski value	0.5
Ester value	149.6	Specific gravity (15°C.)	0.924
Iodine value (Wijs' method)	175.6	Unsaponifiable matter	1.6%
Acetyl value	31.5	Melting point	28~29°C.
Hehner value	89.8		

The fatty oil was saponified by the usual method for about 40 minutes at 60°C, adding to it alcoholic caustic potash. After eliminating the unsaponifiable matter by ether, the saponified liquid was decomposed by H_2SO_4, and the liberatd fatty acids were transferred into ether and washed several times with water until free from mineral acid; then, they were dehydrated by anhydrous Na_2SO_4 and the solvent was removed under diminished pressure. The mixed fatty acids thus obtained were of red brown color, and at ordinary temperature they were solid. The general properties of mixed fatty acids and of the divisions separated into the liquid and the solid by the lead salt ether method are as given in the following Table 2.

Table 2

	Mixed fatty acid	Liquid fatty acid	Solid fatty acid
Solidification point	29.0°C.	——	
Melting point	33~35°C.	——	51~54°C.
Iodine value (Wijs)	191.5	240	12.7
Neutralization value	188 8	182 3	210.3
Mean molecular weight	297.0	307	266 8
Rhodan value		122	
Ether insoluble bromide		84 8%	

(II) Isolation of the Fatty Acids.

First of all, according to the Toyama's method, sodium salt of the highly unsaturated acid, which was soluble in acetone, was filtered from the insoluble salt of the mixed fatty acids; the insoluble part was dissolved by warming in a little quantity of 50% alcohol, and after neutralizing the excess of NaOH by acetic acid, the sodium salt was changed to the lead salt by pouring into the boiling 10% lead acetate; and was separated into two parts—the soluble and the insoluble in ether at ordinary temperature. Then, these parts were decomposed by 20% HCl and transferred as free fatty acids into the petroleum ether (b. p. $<50°$) and freeing from the solvent at low temperature under diminished pressure, each free fatty acid was prepared.

The quantities obtained from the mixed fatty acids were as follows:

(1) The highly unsaturated part, 36%, (2) The lowly unsaturated part, 33%, and (3) The saturated part, 31%. The degrees of the unsaturation and the average molecular weights, of the highly and the lowly unsaturated parts of the acids were as the following:

Table 3

	Highly unsaturated group	Lowly unsaturated group
Neutralization value	178.1	191.0
Mean molecular weight	315 2	293 8
Iodine value (Rosenmund und Kuhnheim's method)	356.0	155.5

(A) Researches of the Highly Unsaturated Part.

25 g. of the fatty acid were changed to its methyl ester under CO_2 atmosphere according to the Haller method, and it was fractionated by distilling in vacuo, and the results obtained were as given in Table 4.

Table 4

No. of fraction	Temp. of air bath	Distilling point		Yield	Iodine value	Ester value
		Temp. °C	Press. mm.			
1	190~200	120~144	0 4	2.2	169 6	200.8
2	200~215	160~173	1.0	4 2	272 4	184 5
3	220	185~193	1.5~2 0	2 8	339.0	176 4
4	227~230	197~205	2 0	6 0	362 1	168.5
5	235~240	205~210	2 0~2 5	7.0	274.0	164 7
Residue				small quantity		

In comparing the constants of each division in the foregoing table with the theoretical ones:—

	Iodine value	Ester value
$C_{18}H_{35-4}O_2\text{-}CH_3$	172.7	190.9
$C_{18}H_{35-6}O_2\text{-}CH_3$	260 8	192.2
$C_{20}H_{39-8}O_2\text{-}CH_3$	319.3	176 4
$C_{22}H_{43-10}O_2\text{-}CH_3$	369.0	163 1

It will be noted that about 60% of the highly unsaturated acids was clupanodonic acid $C_{22}H_{44-10}O_2$, and it is possible to infer that the rest was composed of the fatty acids, namely, $C_{20}H_{40-8}O_2$, $C_{18}H_{36-4}O_2$, ($C_{18}H_{36-4}O_2$?).

Furthermore, to ascertain these facts each division was changed to the reduced methyl ester ($C_nH_{2n-1}O_2$–CH_3), having them reduced in ether by hydrogen, using platinum black as the catalyser; the liberated acids ($C_nH_{2n}O_2$) were separated, the constants estimated, and the results obtained are as shown in Table 5.

Table 5

No. of fraction	Reduced methyl-esters				Freed reduced fatty acids			
	Saponification value		Melting point °C.		Saponification value		Melting point	
	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.
(1)	198.3	188.3 ($C_{18}H_{35}O_2$-CH_3)	26~27	38	195.2	197.3	58	69.3
(2)	180.3		36~37	46~47	178.0		67~68	
(3)	172.5	172.0 ($C_{20}H_{43}O_2$-CH_3)	41~42		173.5	179.8	69~70	75
(4)	167.0		45~46		167.5		74~75	
(5)	160.9	158.5 ($C_{22}H_{43}O_2$-CH_3)	47~48	53~54	194.0	165.0	79~80	79~80

To elucidate—it is inferrable that the division (1), the divisions (2) and (3), and the divisions (4) and (5) were composed of the unsaturated acids corresponding to stearic acid, arachidic acid, and behenic acid respectively.

(B) Researches of the Lowly Unsaturated Part.

(a) By the method of oxidation.

20 g. of the fatty acids were changed by Hazura's oxidizing method to oxidized acids using 3/4 % $KMnO_4$. These acids were extracted with petroleum ether and the unchanged fatty acids eliminated, and the quantity of the oxidized acids thus obtained was found to be about 10 g. This was again extracted for 20 hours with ether and the soluble part was separated from the insoluble.

(1) The soluble part in ether:- The quantity obtained was 4.5 g.; recrystallized twice from 90% alcohol and once from 95 % alcohol; mp. 118°~119°C.; no change of m. p. in recrystallizing from ethyl acetate; the crystal was flat irregular hexagonal; the neutralization value of the acids were 175.7~177.6 (The value as dihydroxy-stearic acid was 177.3).

(2) The insoluble part in ether:- Boiled 8 times in a liter of water for 1~3 hours each. The quantity of the soluble part in hot water was 1.7g., and that of the insoluble part 1.0 g. The former contracted at 120°C., and melted at 166~170°C., and a large part of it dissolved in 90 % alcohol. In crystallizing it formed flat hexagonal crystals; mp. 129~130°C.; the neu-

tralizing point 175.0. The latter is soluble in hot 90 % alcohol and when recrystallized from 95% alcohol, ethyl acetate, m.p. became definite at 119° C; the neutralization value was 174.6. Viz. even though the insoluble part had been extracted for 20 hours with ether, dihydroxy stearic acid still remaining was a large part of it, and tetra hydroxy and hexahydroxy stearic acids might have been present there but the quantities of these acids were so small that their further examinations could not be carried out.

(b) By the method of bromination.

4.2 g. of the fatty acids were treated by the usual method and the bromides were obtained.

(1) The insoluble bromides in ether:- The quantity obtained was 0.5 g.; the content of bromine in the insoluble part in hot benzene was 76.0 % and in the soluble part 71.0 % (The theoretical percentage as $C_{22}H_{34}O_2Br_{10}$ was 70.76%). Thus, it is readily seen that it was the decabromide of clupanodonic acid.

(2) The soluble bromides in ether:- Eliminating the excess of bromine and ether, and in extracting it with petroleum ether, a part became white precipitate. Besides, there was the coating of gelatinous substance on the vessel. The quantity obtained was small; the precipitate white, mp. 113°~115°C.; perhaps, this part might have been the tetrabromides of linolic acid and isolinolic acid.

(3) The soluble part in ether and petroleum ether:- The quantity obtained was 4.2 g. The bromine content was 39 %; the neutralization value was 120; (the theoretical value as the dibromide of oleic acid $C_{18}H_{38}O_2Br_2$–36.18 % Br; the neutralization value 126.9). It is clear that the greater part was the dibromide, though still mixed with tetrabromide.

From the foregoing results, it is seen that a large part of the division of this lowly unsaturated fatty acids was composed of oleic acid, small quantities of linolic acid and the highly unsaturated acids.

(c) Researches of the Saturated Fatty Acids.

20 g. of the fatty acids were made to be their methyl esters by the same method as that used for the division (A), and by distilling twice at the different temperatures under diminished pressure, the divisions obtained are as given in Table 6.

Table 6

The 1st distillation	The 2nd distillation						
	Temp. of air bath	Distilling point: Temp.	Distilling point: Press. mm.	Yield	Melting point°C.	Neutralization value	
I fraction	(1) 160	<140	2.0	0.4	16~19	228.5	Myristic acid theor. 231.8
	(2) 170	146~150	2.0	1.4	18~20	223.7	
0.8 mm.	(3) 173~190	150~160	2.6	1.0	19~22	216.6	
136~147°C.	(4)>190	160	3.0	1.7	26~27	206.7	

II fraction	(1) 175	130	1.0	1.3	23~24	208.7	Palmitic acid
	(2) 180~190	143~148	1.0	4.0	23~24	204.0	theor. 207.8
0.8 mm.	(3) 230	155	1.0	0.3	24	207.6	
150~155°C.	(4)	Residue		0.8		195.1	
III fraction	(1) 183	<140	2.0	0.6	24	202.3	
	(2) 190	145~150	2.0	0.5	24	205.3	
1.0 mm,	(3) 190~200	155~160	2.0	0.4	27~28	199.1	Stearic acid
160~168°C.	(4) 205	165~168	2.0	1.2	27~32	194.2	theor. 188.3
	(5)	Residue		1.7	31~33	186.1	
IV fraction				Small quantity			

From these divisions the liberated fatty acids were prepared, and the results determined of their melting points and neutralization values were as follows:

Table 7

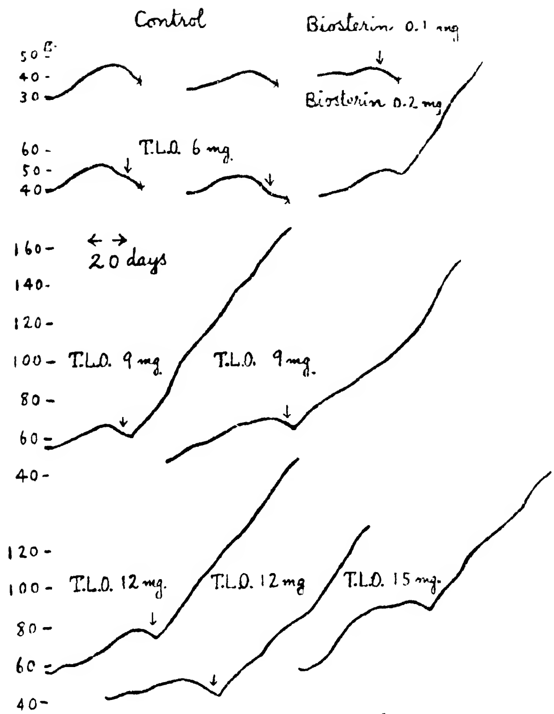
No. of fraction		Melting point °C. Exper.	Melting point °C. theor.	Neutralization value Exper.	Neutralization value theor.
I	(1)	45~50		242.6	245.8 Myristic acid $C_{14}H_{28}O_2$
	(2)	47	53.7	235.5	
	(3)	61~62		220.8	
	(4)	58~59		217.5	
II	(1)	61~62	62.5	217.3	218.9 Palmitic acid $C_{16}H_{32}O_2$
	(2)	62~63		215.1	
	(3)	57~58		213.5	
	(4)	57~60		198.0	
III	(1)	—		—	
	(2)	55~56		211.5	
	(3)	55~56		206 5	
	(4)	68~69	72.0	199.0	197.3 Stearic acid $C_{18}H_{36}O_2$
	(5)	68~69		195.0	

(III) The Vitamin-A Potency of Tunny Liver Oil.

Albino rats weighing about 40 g. were fed with the vitamin-A free diet and, when their weight diminished and xerophtalmia appeared, the tunny liver oil mixed at the rate of 20 % with olive oil was given to them through the mouth. The sample oil was prepared in the manner as follows; the fresh liver of tunny caught in February was dehydrated by anhydrous Na_2SO_4 and was extracted with ether at low temperature. As shown in the diagram in case 0.2 mg. or more of the Biosterin was given to each of the animals per day, they were perfectly cured, while the tunny liver oil showed its complete curative power when 9 mg. or more of it was given.

Considering from the fact that relatively large amount of the Biosterin was required at the present test, it is inferrable that the curative power of the tunny liver oil is approximate to that of a commercial cod liver oil. This may also be verifi ed from the fact that its color reaction by antimony

trichloride is about the same as that of the Shimoda's cod liver oil on market.



T.L.O = Tunny liver oil.

Summary

(1) The isolation and determination of fatty acids of tunny liver oil were performed, and further the examination of the curative value of its vitamin-A was carried out.

(2) The fatty acids contents of the liver oil, collected in spring, were about as follows:

Oleic acid	30%	Clupanodonic acid	22%
Arachidonic acid	20%	Palmitic acid	19%
Stearic acid	7%	Myristic acid	5%

Small amount of Linolic acid.

(3) The curative power of the vitamin of this oil was determined by biological test, and 9 mg. of the oil per day for one albino rat was found to be sufficient.

Before closing this article, the writer wishes to express to Profs. U. Suzuki and M. Yamagawa his sincere acknowledgement and appreciation for their kind direction given him throughout this research work.

Biochemistry of Filamentous Fungi III.

A Metabolic Product of Aspergillus melleus Yukawa. Part II.

By

HIDEJIRO NISHIKAWA.

(Tottori Agricultural College.)

(Received Sept. 6, 1933)

In Part I[1] the writer described a crystalline substance, mellein $C_{10}H_{10}O_3$, which is produced by Aspergillus melleus Yukawa in a medium containing sugar as the sole source of carbon. Further investigation is now reported which may serve to elucidate the structure of mellein.

Action of hot strong nitric acid on mellein produced its dinitro-derivative. Mononitromellein already reported in Part I also gave this substance when further nitrated.

Mellein has no methoxyl. It has a hydroxyl group which is acetylated by means of acetic anhydride and pyridine yielding monoacetylmellein. Monoacetyl-, mononitro-, and dinitromellein are all laevo-rotatory as mellein itself.

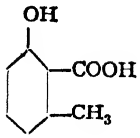
When mellein was submitted to potash fusion at about 200°, it was smoothly converted into an unsaturated acid, which has the same molecular formula, $C_{10}H_{10}O_3$. This acidic isomer of mellein, which is now named melleic acid, is monobasic and has no rotatory power. It absorbed catalytically two atoms of hydrogen giving a saturated acid, $C_{10}H_{12}O_3$.

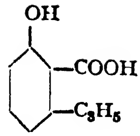
Methylation of melleic acid with diazomethane gave monomethyl ester of melleic acid which gives a violet $FeCl_3$ reaction and is readily reconverted into melleic acid on treatment with alkali. Acetylation with acetic anhydride and pyridine produced its monoacetyl derivative which is a monobasic acid and gives no $FeCl_3$ reaction.

Potash fusion of mellein in the neighbourhood of 300° proceeded with vigorous effervescence and produced along with a trace of cresol a fair amount of an acid which, from analyses, melting points and other properties of itself and its acetyl derivative, is identical with 6-hydroxy-2-methylbenzoic acid (I) obtained by Anslow & Raistrick[2] as a metabolic product of Penicillium griseo-fulvum Dierckx. Melleic acid too was decomposed into this acid when fused with KOH.

From the facts mentioned above the formula of melleic acid will probably be represented by (II).

(1) Bull. Agric. Chem. Soc. Jap., **9**, **107** (1933).
(2) Anslow & Raistrick: Biochem. J., **26**, 43 (1931).


I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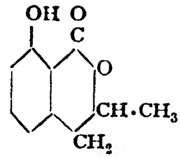
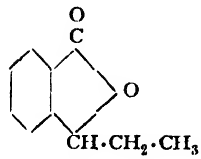

III


IV

To mellein itself structural formulae, (III) or (IV), may be assigned which satisfy the requirements of the facts so far known. Further experimental evidence will however be necessary for definite conclusion.

Both melleic and dihydromelleic acids give violet coloration with $FeCl_3$, colour tone being identical with that of mellein itself and again indistinguishable from that of salicylic acid.

Mellein gives with alcoholic potash beautiful lilac fluorescence.

Experimental.

Dinitromellein.

One gram of mellein was boiled with 10 c.c. conc. HNO_3 (sp. gr. 1.4) under reflux for 2 hours. On cooling, pale yellow needles separated (0.85 g), which after recrystallized from MeOH melted at 160°. (Found: C, 44.73; H, 3.37. $C_{10}H_8O_7N_2$ requires C, 44.78; H, 2.99 %. $[\alpha]_D^{32} = -508°.68$). Further nitration of mononitromellein gave identical dinitro-derivative, m.p. 160° alone or mixed. At one experiment a variety of dinitro compound of m.p. 125°, thin plate crystals from MeOH, was produced; it cannot however be prepared again. (Found: C, 44.38; H, 3.15 %).

Acetylmellein.

1 g of mellein was incubated with 4 c.c. acetic anhydride and 8 c.c. pyridine for 2 days at 35°. When the mixture was diluted with water and acidified with H_2SO_4, crystalline grains separated (0.8 g). Further 0.2 g was cropped from mother liquor by extraction with ether. Recryst. from water, thick hexagonal plates, m.p. 126°. (Found: C, 65.36; H, 5.55. $C_{12}H_{12}O_4$ requires C, 65.45; H, 5.45 %. $[\alpha]_D^{28} = -171°.80$).

Melleic acid.

1 g of mellein was fused with 10 g of KOH at 200°. The fusion proceeded without effervescence. The melt was dissolved in water and saturated with CO_2. Ether extracted practically nothing from it. On acidifying the solution with H_2SO_4 colourless needle crystals separated (0.8g), which melted at 170° after recrystallisation from water. Extraction of the filtrate with ether gave further 0.2 g of impure substance. (Found : C, 67.67; H, 5.78. $C_{10}H_{10}O_3$ requires C, 67.42; H, 5.62 %. Molecular weight estimated by titration. Found : 178.9. Calc.: 178). Melleic acid closely resembles salicylic acid in appearance.

Dihydromelleic acid.

0.5 g of melleic acid was catalytically hydrogenated in an ether solution, 0.2 g Pd–$BaSO_4$ being used as catalyst. The reaction finished in a few minutes. Residue from the ether (yield theoretical), when recrystallized from water, separated in colourless needles similar to melleic acid. M.p. 116°. (Found: C, 66.71; 6.78. $C_{10}H_{12}O_3$ requires C, 66.67; H, 6.67 %).

Melleic acid methyl ester.

An excess of diazomethane in ether solution was added to the solution of melleic acid in the same solvent. After immediate brisk evolution of gas ceased, ether driven off, the residue, for purification, was dissolved in alcohol, cooled in ice, and precipitated by adding ice-cold water. It has weak but characteristic smell. (Found: C, 68.72; H, 6.52. $C_{11}H_{12}O_3$ requires C, 68.75; H, 6.25 %). Dihydromelleic acid gave a liquid derivative when treated with diazomethane as above. Mellein did not react with diazomethane under the same experimental condition. Melleic acid was regenerated when the methyl ester was hydrolysed by warming with dilute NaOH and then acidified. The substance obtained melted at 170°, alone or mixed with an authentic specimen of melleic acid.

Acetylmelleic acid.

0.5 g of melleic acid was incubated at 35° with 2 c.c. acetic anhydride and 4 c.c. pyridine for 4 days, The mixture was cooled, diluted with water, acidified with H_2SO_4 and extracted with ether. The syrupy residue from the ether solidified to a crystalline magma while left in vacuum over KOH. Colourless angular grains, freed from mother liquor on porous porcelain, melted at 110°. No suitable solvent for recrystallization having been found, the substance was analysed without further purification. (Found: C, 65.02; H, 5.53. $C_{12}H_{12}O_4$ requires C, 65.45; H, 5.45 %). Titration with $N/10$ NaOH gave an equivalent of 223.3, assuming this to be a monobasic acid. Theoretical 220. An excess of $N/10$ NaOH was added to neutralised solution of the acid, the mixture boiled for 2 hours under reflux, cooled, and excess of alkali titrated with $N/10$ HCl. Acidity equivalent to monoacetyl was produced during hydrolysis.

6–Hydroxy–2–methylbenzoic acid.

1 g of mellein was submitted to potash fusion with 10 g of KOH at 300～310°. Vigorous evolution of gas took place. The fused mass was dissolved in water, saturated with CO_2, and extracted with ether. When the ether was driven off, a minute quantity of oily matter remained having strong smell of cresol. The solution after being acidified was again extracted with ether. The residue from the ether (0.7 g) crystallized from water in colourless needles, m.p. 170°. (Found: C, 62.90; H, 5.45. $C_8H_8O_3$ requires C, 63.16; H, 5.26 %. Mol. wt, by titration 150. Calc. 152). The substance

described by Anslow & Raistrick has m.p. 170~171°. Other properties are also identical.

Acetyl derivative of 6-hydroxy-2-methylbenzoic acid.

0.4 g of 6-hydroxy-2-methylbenzoic acid, 2 c.c. acetic anhydride and 4 c.c. pyridine were mixed and incubated at 30° for 3 days. The mixture was diluted with H_2O, acidified with H_2SO_4, and extracted with ether. The residue from the ether crystallized from benzene in colourless prisms (1st crop 0.2 g), m.p. 131°. (Found: C, 61.89; H, 5.29. $C_{10}H_{10}O_4$ requires C, 61.85; H, 5.15%). Titration with N/10 NaOH to phenolphthalein gave an equivalent of 194.6. Theoretical 194. Boiling with an excess of N/10 NaOH and back titration with N/10 HCl showed that acidity equivalent to monoacetyl was produced during the hydrolysis. Acetyl derivative prepared by Anslow & Raistrick melts at 131°.

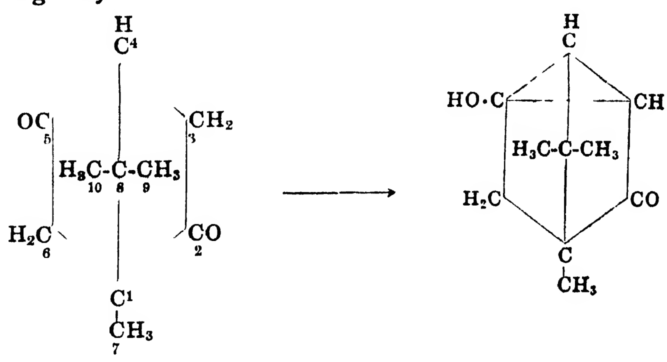
On the Physiologically Active Isomer of Bredt's 5-Oxo-camphor.

By

Kunijiro TAKEUCHI and Yoshikazu SAHASHI.

(Received August 29, 1933)

During the studies on the camphor group the present authors have observed that the Bredt's 5-oxo-camphor, prepared from borneol(1) can be transformed into its so-called enol-isomer by prolonged boiling with hexane in the following way:



Bredt's 5-oxo-camphor (prismatic crystal)

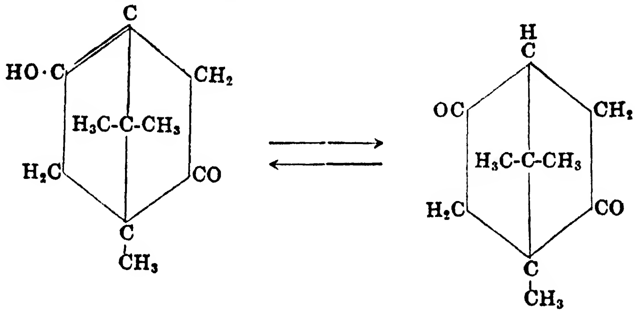
Enol-isomer (irregular plate)

The prismatic crystals are converted thereby into irregular plates and at the same time acquire the property of stimulating the heart of animals

while the keto-isomer has no such activity.

This observation led the authors to examine the so-called allo-*p*-oxo-camphor prepared by Tamura, Asahina and co-workers,[2] to determine whether or not the active component in the said preparation is identical with the enol-isomer mentioned above. For this purpose, 5-oxy-camphor was first prepared from the urine of dogs administered with camphor per os and it was converted into allo-*p*-oxo-camphor by carefully oxidizing with sulphuric acid and sodium bichromate, following the method of above authors.

This preparation is stated by these authors to be the mixture of keto- and enol-isomers of the following formulae:



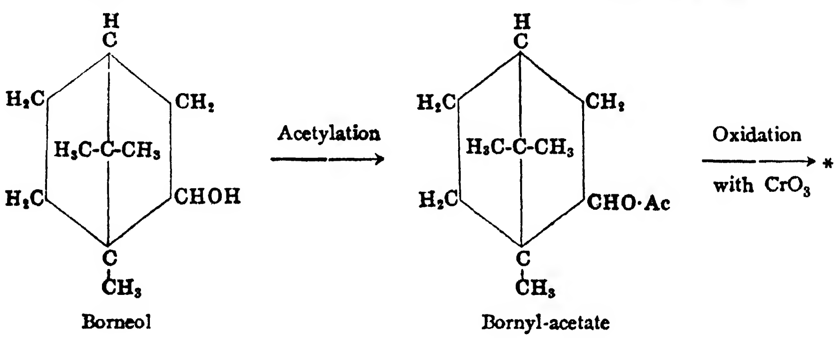
(Allo-*p*-oxo-camphor)

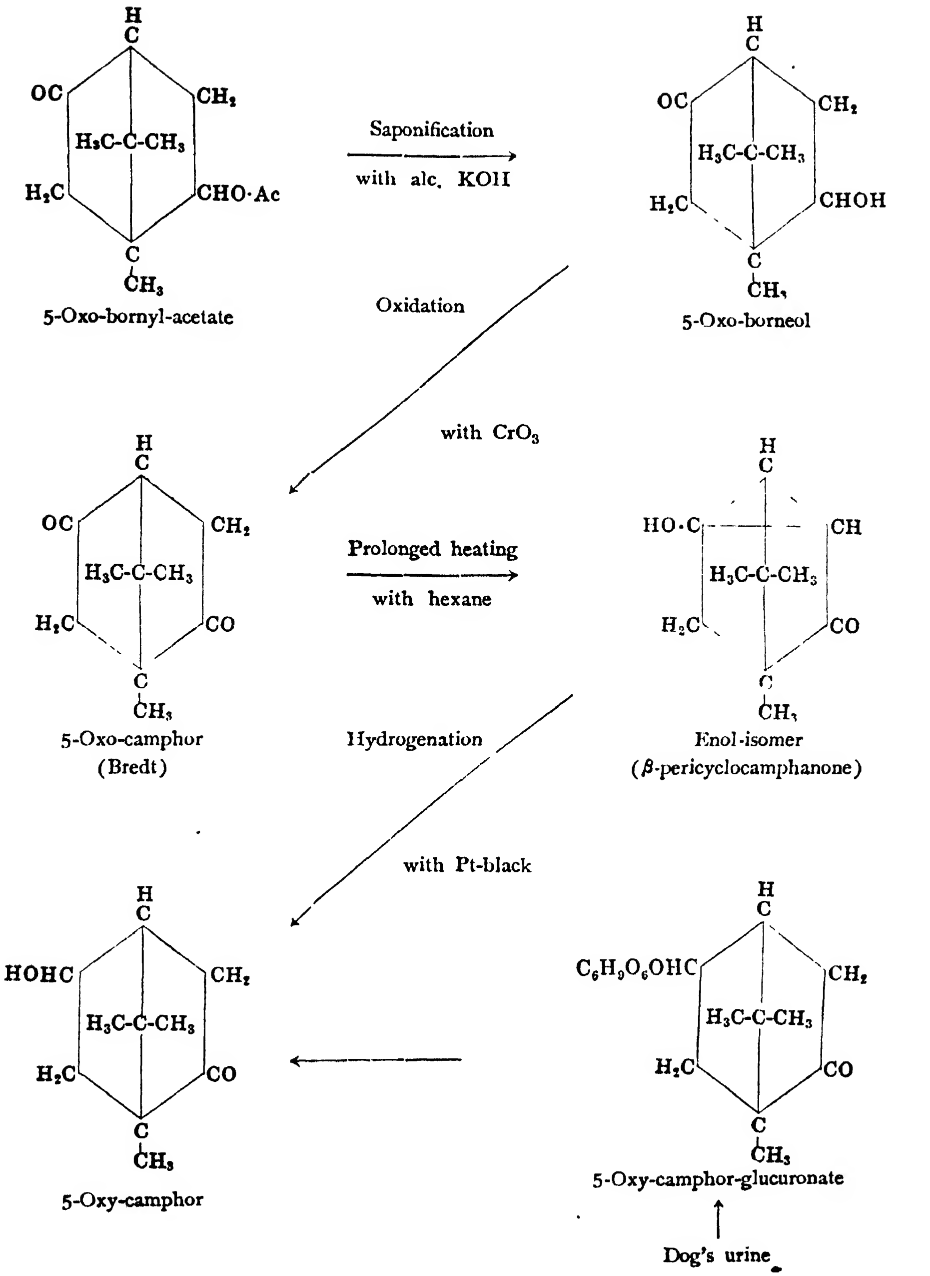
Under the microscope, it was revealed to be the mixture of two kinds of crystals, i. e. the prisms and irregular plates as stated by these authors.

The present authors have now observed that by prolonged boiling with hexane the prismatic crystals in the above preparation were gradually converted into plate ones and the latter was exactly identical, both in its chemical properties as well as in its physiological behaviours, with the enol-isomer of the synthetic 5-oxo-camphor, the stimulating action upon the heart of animals being estimated to be quite similar in both cases.[3]

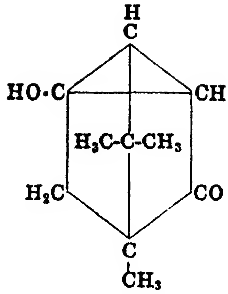
Further, the present authors have regenerated 5-oxy-camphor by reducing the enol-isomer of the synthetic 5-oxo-camphor and proved it to be identical with the preparation, isolated from dog's urine according to the method of Asahina and co-workers.

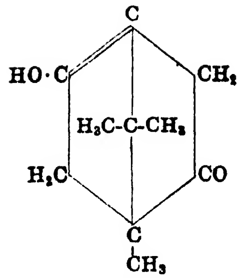
This relation may be briefly stated in the following scheme:





The question, whether the enol-isomer mentioned above is represented by the formula (I) or (II) remains to be settled.

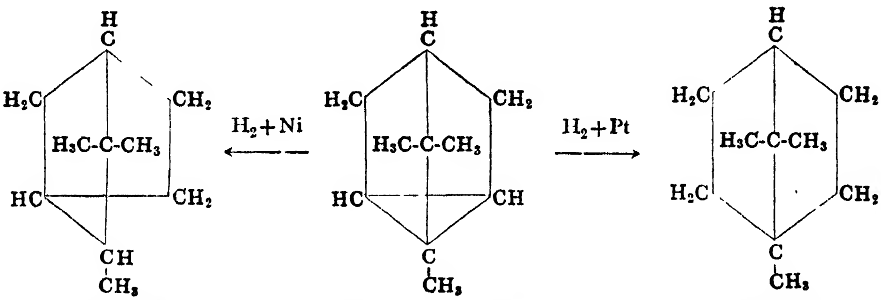

(I)


(II)

The present authors are however inclined to accept the formula (I) from the following reasons:

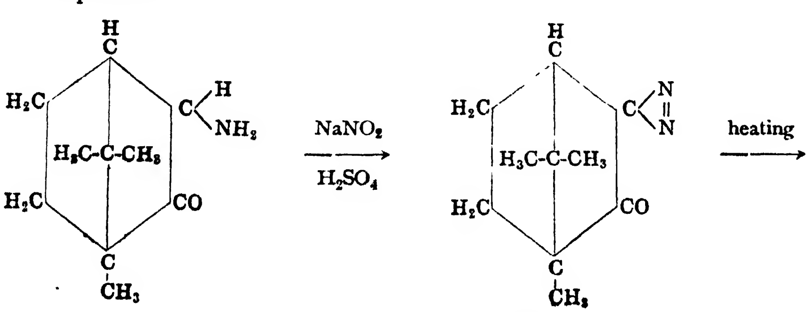
(1) There is no absorption spectra or any chemical reaction indicating the presence of double bond.

(2) 5-Oxo-camphor of keto form (prismatic crystals) cannot be hydrogenated with hydrogen and platinum while the enol-isomer is slowly reduced to 5-oxy-camphor.[4] This is in analogy with the fact that *α*-pericyclocamphane can be reduced to camphane by hydrogen and platinum and to isocamphane by hydrogen and nickel.

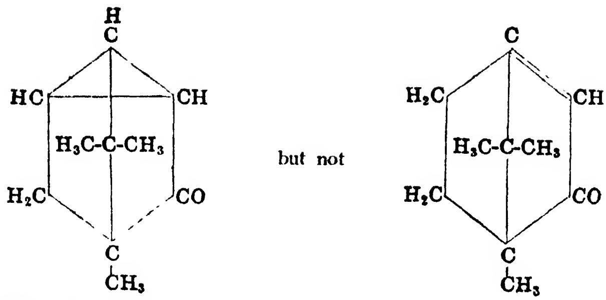


Iso-camphane   *α*-Pericyclo-camphane (*α*-Tricyclene)   Camphane

(3) Bredt and Holz[5] proved experimentally that diazocamphor derived from *α*-amino-camphor does not form camphenone by heating but gives *β*-pericyclocamphanone.

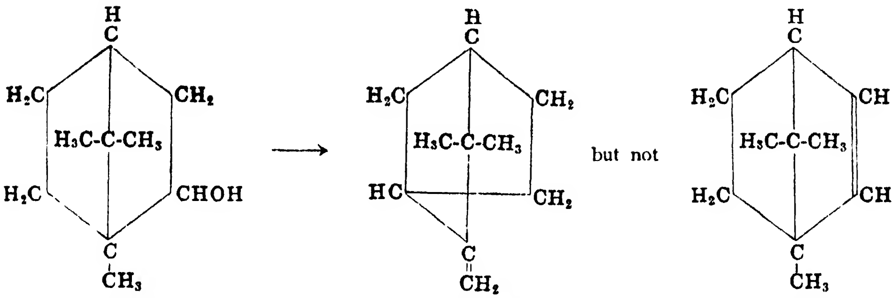


α-Aminocamphor   Diazocamphor



β-Pericyclo-camphanone but not Camphenone

Further it is well known that borneol is transformed to camphene by dehydrating agents but not to bornylene.



Borneol → Camphene but not Bornylene

So the existence of double bond in the enol-isomer of 5-oxo-camphor is very improbable and the authors believe that the formula (I) (tricyclene form) is in better accordance with various facts.

Experimental

I. *Synthetical Preparation of 5-Oxo-camphor from Borneol after Bredt.*

(1) 5-Oxo-borneol: Bornyl-acetate [b p/4 mm = 80~81° (uncorr.)] was first prepared from borneol by heating with acetic anhydride and it was oxidized into 5-oxo-bornyl-acetate [b p/4~5 mm = 125~127° (uncorr.)] with chromic anhydride in glacial acetic acid. The latter was then saponified with alcoholic potash in the usual way and after evaporating off the alcohol, the residual solution was saturated with CO_2 and extracted with ether. The 5-oxo-borneol thus obtained was recrystallized from alcohol; m p = 232~235° (uncorr.), $[\alpha]_D^{18.0°} = +82.1°$ in alcohol (c = 2.46), (Bredt's 5-oxo-borneol; m p = 238~246° (uncorr.), $[\alpha]_D^{14.5°} = +71°$).

The authors are indebted to Messers T. Iki and K. Tanaka for analyses.

Analysis: 3.781 mg subs. gave 9.919 mg CO_2, 3.274 mg H_2O;
C = 71.55%, H = 9.69%.
4.021 " " " 10.564 " ", 3.509 mg H_2O;
C = 71.65%, H = 9.76%.
Calc. for $C_{10}H_{16}O_2$ C = 71.37%, H = 9.59%.

(2) 5-Oxo-camphor: This was obtained by oxidizing the 5-oxo-borneol, mentioned above, with chromic acid in boiling water-bath and was recrystallized from ligroin in prismatic crystals (Photo. 5); m p = 207°(uncorr.), $[\alpha]_D^{17.0°} = +103.5°$ in ligroin (c = 2.025). (Bredt's 5-oxo-camphor; m p = 206.5 ~206.7°, $[\alpha]_D^{13.5°} = +103°$).

Analysis: 3.882 mg subs. gave 10.282 mg CO_2, 2.950 mg H_2O;
C = 72.23%, H = 8.50%.
4.111 " " " 10.870 " ", 3.137 mg H_2O;
C = 72.11%, H = 8.54%.
Calc. for $C_{10}H_{14}O_2$ C = 72.28%, H = 8.44%.

(3) The enol-isomer of 5-oxo-camphor: When 5-oxo-camphor (prismatic crystals) obtained as above was boiled with ligroin or hexane for a long time,* it was converted into its enol-isomer, which crystallized in colourless irregular plates (Photo. 6).

The preparation treated with ligroin: m p = 209~210° (uncorr.); $[\alpha]_D^{17.0°} = +144.6°$ in ligroin (c = 2.109).

The preparation treated with hexane: $[\alpha]_D^{17.0°} = +147.3°$ in ligroin (c = 1.988).

The preparation purified from ligroin, was analyzed.

Analysis: 4.040 mg subs. gave 10.688 mg CO_2, 3.150 mg H_2O;
0.1993 g subs. 26.92 g benzol gave Δ = 0.22;
C = 72.15%, H = 8.72%, mol. wt. = 168.
Calc. for $C_{10}H_{14}O_2$ C = 72.28%, H = 8.44%, mol. wt. = 166.

(4) Semicarbazone of the enol-isomer: When the enol-isomer, mentioned above, was treated in the usual way with semicarbazide hydrochloride and Na-acetate and the reaction product was recrystallized from alcohol, the mono-semicarbazone was obtained as colourless needles; soluble in alcohol, m p = 230~231° (uncorr.).

Analysis: 3.810 mg subs. gave 0.6070 c.c. N_2 (758.5 mm, 19°C);
N = 18.60%.
3.445 " " " 0.5468 " N_2 (762.5 mm, 18.5°C);
N = 18.56%.
Calc. for $C_{11}H_{17}O_2N_3$ N = 18.80%.

* We have also prepared the above enol-isomer from Bredt's prismatic 5-oxo-camphor by heating at 200°C in a sealed tube, which was previously evacuated by means of high vacuum pump.

It was observed that the above enol-isomer had the activity upon the heart of animals similar to the preparation obtained from dog's urine.

II. *Regeneration of 5-Oxy-camphor by the Reduction of the Enol-isomer of 5-Oxo-camphor.*

(1) Five grams of enol-isomer of 5-oxo-camphor, synthetically prepared from borneol, were dissolved in 5 g acetic acid and treated with hydrogen gas for 50～100 hours, using platinum-black as catalyst. The solution was then diluted with water, filtered from the catalyzer, neutralized with sodium carbonate and extracted with ether. The etherial solution was evaporated to dryness and the crystalline residue thus obtained was treated with 10 % caustic potash and converted into acetate. By saponifying the latter, 5-oxy-camphor was regenerated. When recrystallized from large amount of ligroin it formed nice characteristic crystals (Photo. 7); m p = 220～222° (uncorr.), $[\alpha]_D^{18.0°} = +18.5 \sim 50.2°$ in ligroin, $[\alpha]_D^{18.5°} = +47.5°$ in alcohol ($c = 2.862$). Mixed with pure specimen of 5-oxy-camphor (m p = 222.5°, $[\alpha]_D^{18.5°} = +47.6°$) prepared from dog's urine, no depression of melting point was observed.

Analysis: 4.340 mg subs. gave 11.295 mg CO_2, 3.679 mg H_2O;
C = 70.98%, H = 9.48%.
4.025 〃 〃 〃 10.510 〃 〃, 3.430 mg H_2O;
C = 71.21%, H = 9.53%.
Calc. for $C_{10}H_{16}O_2$ C = 71.37%, H = 9.59%.

(2) 5-Oxy-camphor-semicarbazone was prepared from the above sample in the usual way; m p = 220° (uncorr.).

Analysis: 3.960 mg subs. gave 0.5998 c.c N_2 (770 mm, 17.5°C);
N = 18.03%.
Calc. for $C_{11}H_{19}O_2N_3$ N = 18.6%.

(3) 5-Acetoxy-camphor was prepared from the same sample by heating with acetic anhydride in the usual method and purified by distilling in vacuum; colourless liquid, b p (25 mm) = 137～140°C (uncorr.).

Analysis: 5.054 mg subs. gave 12.730 mg CO_2, 3.982 mg H_2O;
C = 68.69%, H = 8.81%.
5.018 〃 〃 〃 12.633 〃 〃, 3.910 mg H_2O;
C = 68.66%, H = 8.71%.
Calc. for $C_{12}H_{18}O_3$ C = 68.57%, H = 8.5%.

(4) 5-Acetoxy-camphor-semicarbazone was prepared from the above acetate; m p = 197～200°C (uncorr.).

Analysis: 3.476 mg subs. (dried at 100°C) gave 7.338 mg CO_2, 2.578 mg H_2O;
C = 57.57%, H = 8.28%.
3.648 " " (dried at 150°C) " 7.789 " ", 2.660 mg H_2O;
C = 58.17%, H = 8.15%.
Calc. for $C_{13}H_{21}O_2N_3$ C = 58.42%, H = 7.90%.
3.040 mg subs. (dried at 150°C) gave 0.4185c.c. N_2 (763 mm, 28°C);
N = 15.63%.
2.877 " " (dried at 150°C) " 0.4057c.c. N_2 (759 mm, 28°C);
N = 15.97%.
Calc. for $C_{13}H_{21}O_2N_3$ N = 15.73%.

III. *Isolation of 5-Oxy-camphor from Dog's Urine.*

(1) 5-Oxy-camphor was isolated from dog's urine according to the method of Y. Asahina and M. Ishidate, and after boiling with 10% caustic potash for an hour, it was converted into the acetate. The latter was then saponified with alcoholic potash in the usual way and recrystallized from ligroin (Photo. 10):

m p = 222.5°C (uncorr.); $[\alpha]_D^{24.0°} = +43.9°$ in abs. alcohol (c = 2.956) or
$[\alpha]_D^{18.5°} = +47.1°$ in abs. alcohol (c = 2.564).

Analysis: 3.792 mg subs. gave 9.842 mg CO_2, 3.330 mg H_2O;
C = 70.79%, H = 9.82%.
4.500 " " " 11.700 " ", 3.878 mg H_2O;
C = 70.91%, H = 9.64%.
Calc. for $C_{10}H_{16}O_2$ C = 71.37%, H = 9.59%.

(2) 5-Acetoxy-camphor was prepared from the above sample; b p (25 mm) = 150~151°C (uncorr.).

Analysis: 4.444 mg subs. gave 11.141 mg CO_2, 3.418 mg H_2O;
C = 68.37%, H = 8.60%.
Calc. for $C_{12}H_{18}O_3$ C = 68.57%, H = 8.5%.

(3) 5-Acetoxy-camphor-semicarbazone; m p = 180~190°C (uncorr.).

Analysis: 4.210 mg subs. gave 8.964 mg CO_2, 3.047 mg H_2O;
C = 58.07%, H = 8.09%.
4.088 " " " 9.5468c.c. N_2 (764 mm, 22.5°C);
N = 15.53%.
Calc. for $C_{13}H_{21}O_2N_3$ C = 58.42%, H = 7.90%,
N = 15.73%.

The above data agree fairly well with those reported by Y. Asahina and M. Ishidate* as shown in the following Table:

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* We have also isolated 5-oxy-camphor form the urine of rabbits administered with camphor per os, and confirmed it to be identical with the preparation obtained from dog's urine.
m p = 215~217°C (uucorr.).
Analysis: 3.325 mg subs. gave 8.660 mg CO_2, 2.880 mg H_2O;
C = 71.04%, H = 9.69%.
3.483 " " " 9.088 " " 3.024 mg H_2O;
C = 71.16%, H = 9.71%.
Calc. for $C_{10}H_{16}O_2$ C = 71.37%, H = 9.5%.

	Present authors	Y. Asahina and M. Ishidate
5-Oxy-camphor	m p = 222.5°C; $[\alpha]_{D}^{24,0°}$ = +43.9° in alcohol; C = 70.85%, H = 9.73%.	m p = 222°C; $[\alpha]_{D}^{30,0°}$ = +43 2° in alcohol; C = 70.9%, H = 9.7%.
5-Acetoxy-camphor	b p (25 mm) = 150∼151°C; C = 68.37%, H = 8.60%.	b p (27 mm) = 158∼160°C.
5-Acetoxy-camphor-semicarbazone	m p = 180∼190°C; C = 58.07%, H = 7.90%. N = 15.53%.	m p = 180∼185°C; N = 16.01%.

IV. *Oxidation of 5-Oxy-camphor.*

5-Oxo-camphor: The purified 5-oxy-camphor mentioned above was converted into 5-oxo-camphor by oxidizing with sulphuric acid and potassium bichromate at 50∼60° in the same way as reported by Asahina and Ishidate and recrystaliized from hexane; m p = 197∼200° (uncorr.), $[\alpha]_{D}^{[illegible]}$ = + 70.8° in abs. alcohol (c = 2.988).

Analysis: 3.288 mg subs. gave 8.640 mg CO_2, 2.770 mg H_2O;
C = 71.66%, H = 9.42%
Calc. for $C_{10}H_{14}O$ C = 72.28%, H = 8.44%.

The 5-oxo-camphor thus obtained was revealed to be the mixture of two kinds of crystals i. e. the prisms and irregular plates (Photo. 8) but on prolonged boiling with hexane, the prismatic crystals were gradually converted into plate form (Photo. 9); m p = 195∼200°C (uncorr.), $[\alpha]_{D}^{[illegible]}$ = + 101.6° in alcohol (c = 3.266).

Analysis: 3.683 mg subs. gave 9.635 mg CO_2, 3.030 mg H_2O,
C = 71.35%, H = 9.20%.
Calc. for $C_{10}H_{14}O_2$ C = 72.28%, H = 8·44%

The stimulant action of the above compounds upon the heart of animals were determined according to Langendorff's method, and the results are shown in the following Photos (Photo. 11∼20).

The authors wish to express their sincere thanks to Professors U. Suzuki, T. Shimamura and T. Yabuta for their kind advices throughout this work. Thanks are also due to Dr. S. Kato and Mr. T. Shimamoto for their kind help.

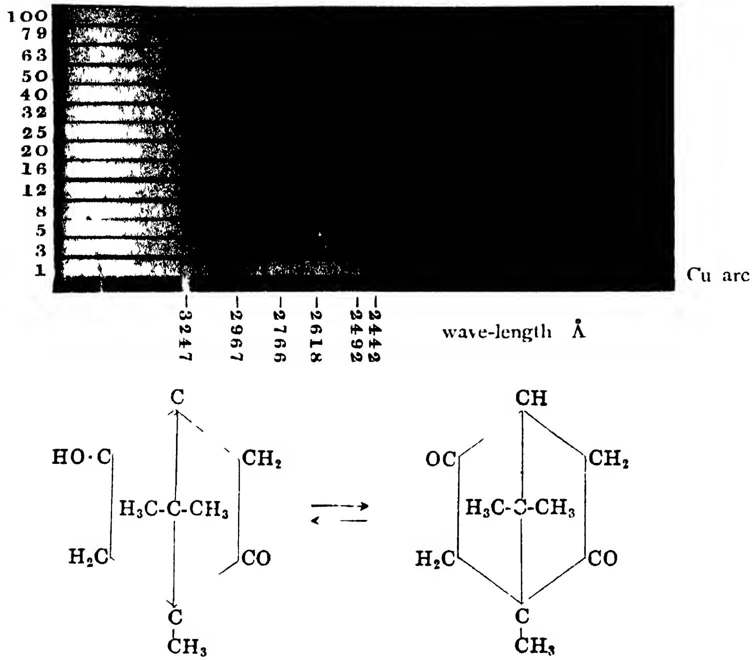


Photo. 1—Commercial Vitacamphor
(0 0825 g in 50 c.c. water)

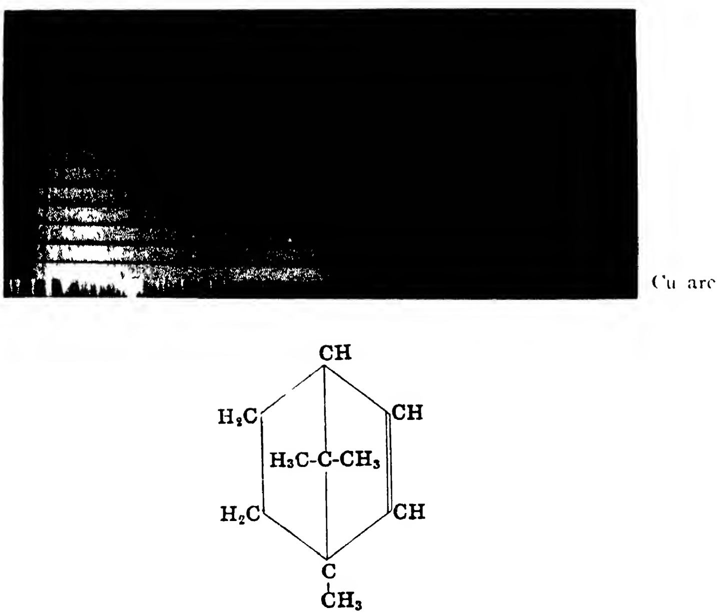
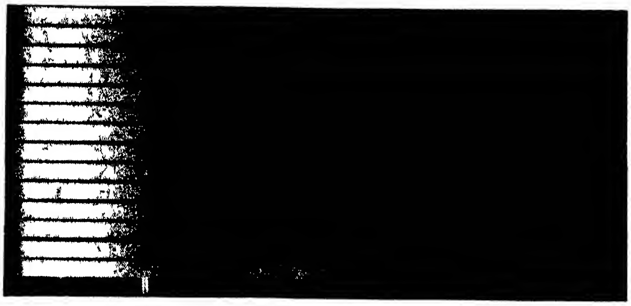


Photo. 2—Bornylene (0.0094 g in 50 c c. ether).



Cu arc

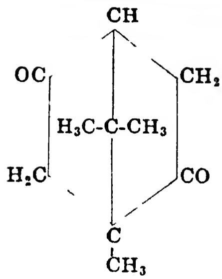


Photo. 3—Bredt's 5-oxo camphor (prismatic form)
(0 0830 g. in 50 c.c. ether)

Cu arc

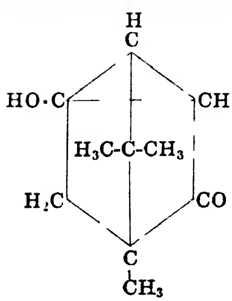


Photo. 4—Synthetic enol form of 5-oxo-camphor
(plate form)
(1/100 mol ether)

Photo. 5—Bredt's 5-oxo-camphor (prismatic form)

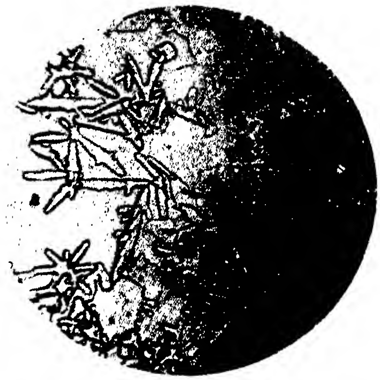


Photo. 8—5-Oxo-camphor obtained from 5-oxy-camphor in dog's urine (the mixture of two kinds of crystals).

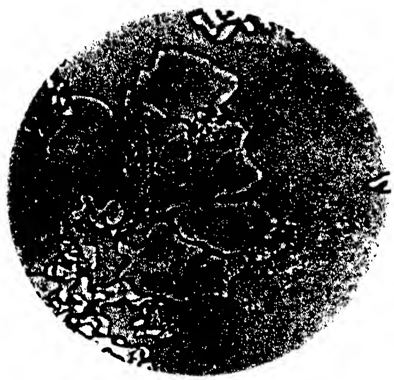


Photo. 6—Enol-isomer . of Bredt's 5-oxo camphor (plate form).

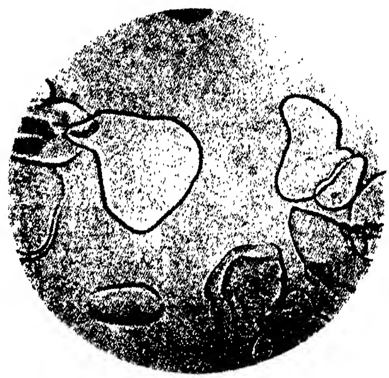
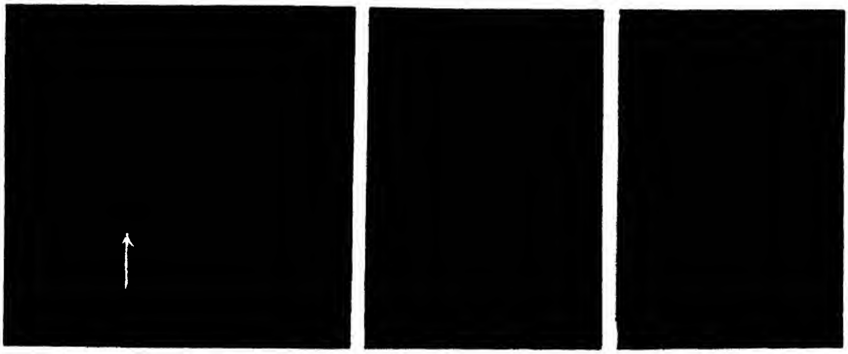


Photo. 9—Enol-isomer of 5-oxo-camphor from 5-oxo-camphor in pog's urine.

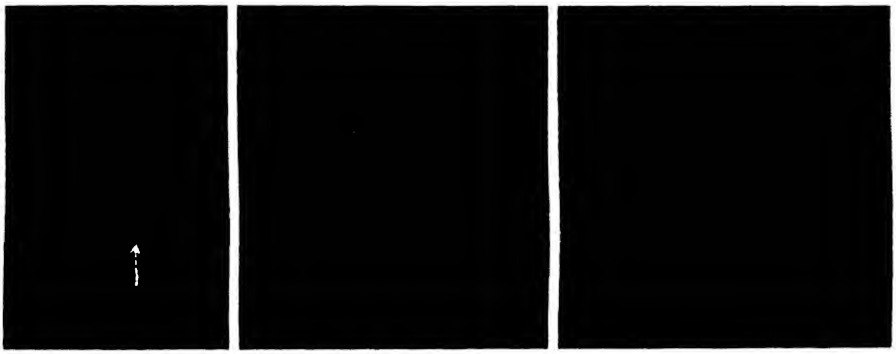
Photo. 7—Synthetic 5-oxy-camphor.

Photo. 10—5-Oxy-camphor obtained from dog's urine.



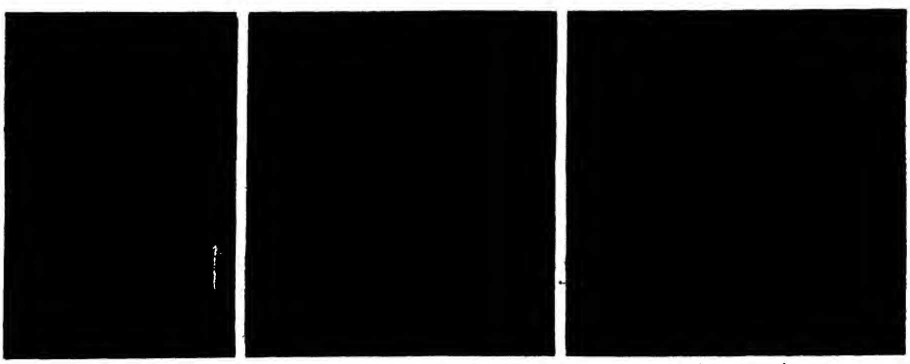
after 5 min after 22 min

Photo. 11—Showing the action of synthetic 5-oxo-camphor (m p 207°, $[\alpha]_D^{17^\circ} = +103$) 0.1%, 0.5 c c, upon the heart of guinea pig.



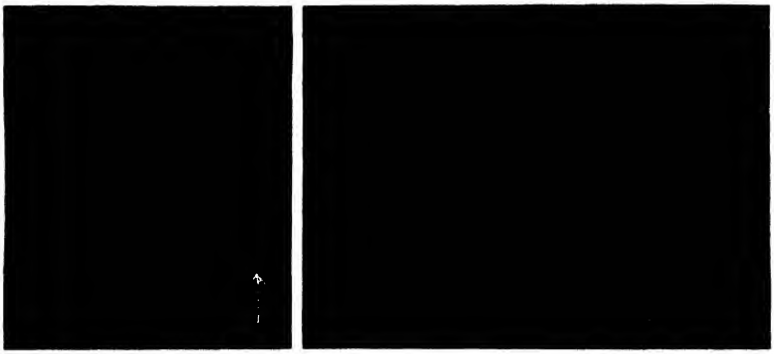
after 8 min after 20 min

photo. 12—Stimulant action of synthetic 5-oxo-camphor (plate form; m p 210°, $[\alpha]_D = +144.6^\circ$) upon the heart of guinea pig; 0.1%, 0.5 c.c.



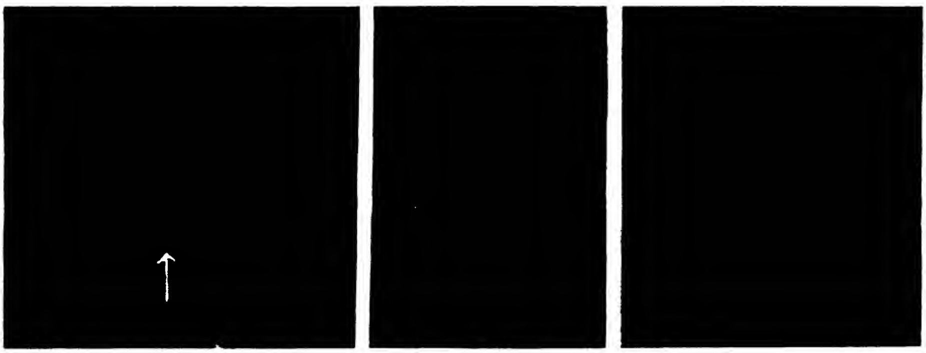
after 10 min after 18 min

Photo. 13—Stimulant action of synthetic 5-oxo-camphor (plate-form; m p 210°, $[\alpha]_D = +144.6^\circ$) upon the heart of guinea pig; 0.5%, 0.2 c.c.



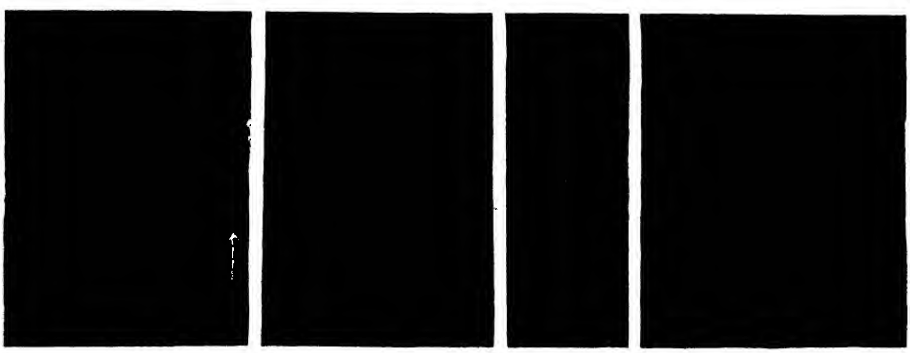
after 18 min

Photo. 14—Stimulant action of synthetic 5-oxo-camphor (plate-form; m p 210°, $[\alpha]_D = +144.6°$) upon the heart of rabbit; 0.5%, 0.4 c.c.



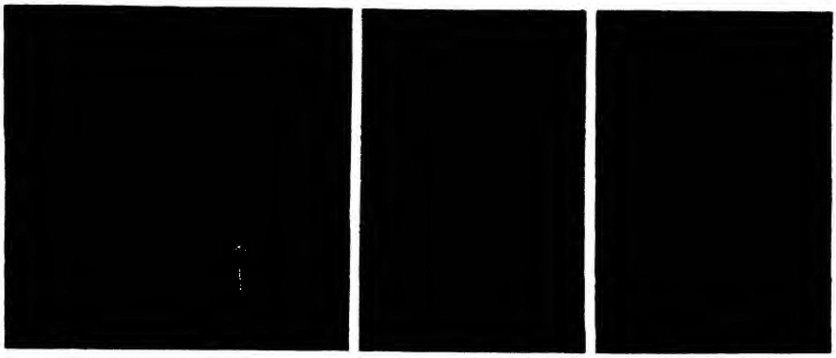
after 5 min after 12 min

Photo. 15—Stimulant action of synthetic 5-oxy-camphor (m p 220~221°, $[\alpha]_D = +18°$) upon the heart of guinea pig; 0.5 %, 0.2 c.c.



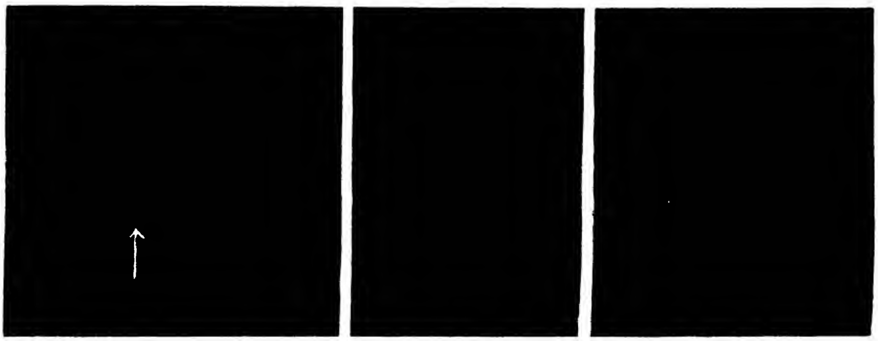
after 8 min after 40 min

Photo. 16—Stimulant action of synthetic 5-oxy-camphor (m p 220~221°), $[\alpha]_D = +18°$) upon the heart of guinea pig; 0.5 %, 0.5 c.c.



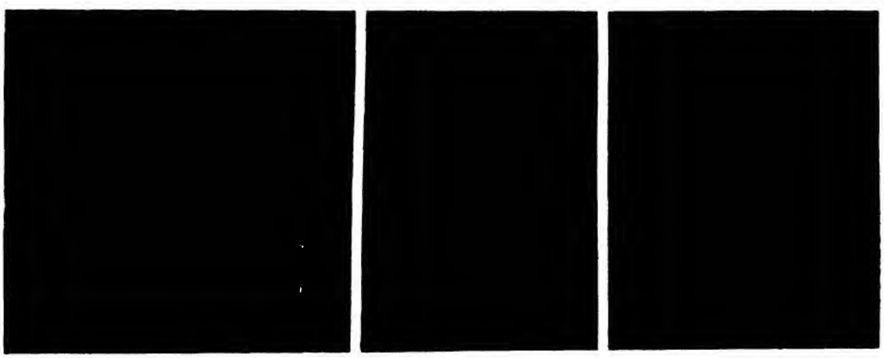
after 8 min after 20 min

Photo. 17—Stimulant action of 5-oxy-camphor from dog's urine (m p 222.5°, $[\alpha]_D = +43.9°$) upon the heart of rat; 0.5%, 0.5 c.c. aq.



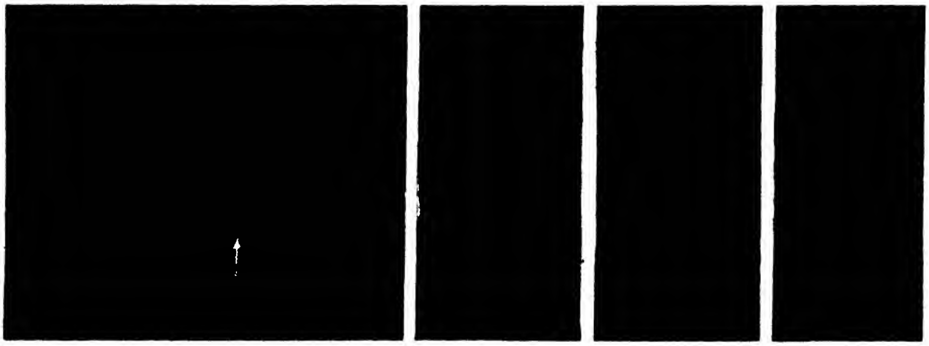
after 12 min after 30 min

Photo. 18—Stimulant action of 5-oxo-camphor from dog's urine (mixture of plate and prisms; m p 195~200°C, $[\alpha]_D = +70°$) upon the heart of guinea pig; 0.5%, 0.2 c.c.



after 7 min after 17 min

Photo. 19—Stimulant action of 5-oxo-camphor from dog's urine (plate form; m p 195~200°C, $[\alpha]_D = +101.6°$) upon the heart of rat; 0.5%, 0.5 c.c.



after 10 min after 30 min after 60 min

Photo. 20—Stimulant action of 5-oxo-camphor from dog's urine (plate form; m p = 195～200°, $[\alpha]_D = +101.6°$) upon the heart of guinea pig; 0.5%, 0.2 c.c.

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On the Organic Bases, especially Agmatine of "Di-Saké".

By

Kotaro NISHIDA and Aritomo YAMADA

(*Kagoshima Agricultural College, Kagoshima Japan*)

(Recieved July 6, 1933)

Recently one of the authors (Bull. Agr. Chem. Soc. Japan, Vol. 8, Nos. 10～12, 151) reported that the occurrence of hypoxanthine, arginine, choline and ammonia in "di-saké", which is a kind of "saké" resulting after pressing the fermented rice.

In this experiment, the authors isolated hypoxanthine, choline, ammonia, betaine and agmatine[1] from "di-saké".

Experimental part.

Experiment 1.

For the isolation of the organic bases 20 litres of the "di-saké" were evaporated under reduced pressure to a small volume, almost equal to 1/3 of the original. After this operation the protein substance and other impurities were removed by lead acetate, and excess of the lead by H_2SO_4, and then the organic bases were precipitated by phosphotungstic acid. And according to the general method the precipitate formed by phosphotungstic acid was fractionated into three fractions, and in each fraction researches were made about the organic bases.

(1) *Purine base-fraction*, (*hypoxanthine*)

The yield of the base from this fraction was 0.60 g. as hydrochloride. On analysing this hydrochloride, the following result was obtained:

0.1196 g. subst.	0 03483 g. N	29 12% N
Calc. for $C_5H_4N_4O \cdot HCl \cdot H_2O$ (Hypoxanthine hydrochloride)		29.41% N

The chloroaurate of the base formed yellow prisms, decomposed at 248°C.

0.1106 g. subst.	0.0453 g. Au	40.96% Au
0.0871 g. subst.	0.0358 g. Au	41.10% Au
Calc. for $C_5H_4N_4O \cdot HCl \cdot AuCl_3$ (Hypoxanthine chloroaurate)		41.42% Au

(2) *Arginine-fraction* (*agmatine*)

The yield of the base from this fraction was 1.40 g. as nitrate.

The nitrate of the base, easily soluble in water and given strong Sakaguchi's reaction, crystallized in bright colourless thin leaflets, and melted at 150～151°C.

Its analytical results agreed with the nitrate of the compound, which has the formula $C_5H_{14}N_4$.

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%
(1)	3.233	2.840	1.878	23.96	6.49	——
(2)	2.963	2.600	1.720	23.93	6.49	——
(3)	3.486	3.048	2.040	23.90	6.53	——
(4)	5.217	1.4504 c.c. N (23.0°C., 763.0 m.m.)				32.19
(5)	3.307	0.9261 c.c. N (23.5°C., 763.5 m.m.)				32.39
Calc. for $C_5H_{14}N_4 \cdot 2HNO_3$				23.42	6.30	32.81

The nitrate was converted into picrate by adding Na-picrate to its aqueous solution. The picrate formed deep yellow prisms, hardly soluble in water, and decomposed at 239°C.

[illegible] g. subst.	0.0755 g. nitronpicrate	77.55% picric acid
Calc. for $C_5H_{14}N_4 \cdot 2C_6H_3N_3O_7$ (Agmatine picrate)		77.88% picric acid

The chloroaurate of the base was prepared from the hydrochloride, which was obtained by decomposing the picrate by HCl.

0.2387 g. subst.	0.1166 g. Au	48.85% Au
0.3251 g. subst.	0.1593 g. Au	49.00% Au
Calc. for $C_5H_{14}N_4 \cdot 2HCl \cdot 2AuCl_3$ (Agmatine chloroaurate)		48.67% Au

The chloroplatinate of the base, easily soluble in water, crystallized in orange yellow prisms and decomposed at 216°C.

0.1152 g. subst.	0.0420 g. Pt	36.46% Pt
Calc. for $C_5H_{14}N_4 \cdot 2HCl \cdot PtCl_4$ (Agmatinechloroplatinate)		36.14% Pt

From these results, the organic base obtained from the arginine-fraction of "di-saké" is no doubt agmatine.

(3) *Lysine-fraction* (*betaine and choline*)

The hydrochloride obtained by this fraction, was treated with absolute alcohol and separated into two portions.

(a) Insoluble portion by absolute alcohol: Yield, 0.80 g. It was colourless short prisms, and the derivatives were prepared as follows:

The picrate formed greenish yellow prisms and melted at 181°C

The chloroaurate of the base, hardly soluble in water, crystallized in golden yellow plates of pearly lustre and decomposed at 242°C.

0.2279 g. subst.	0.0983 g. Au	43.13% Au
0.1973 g. subst.	0.0849 g. Au	43.03% Au
Calc. for $C_5H_{11}NO_2 \cdot HCl \cdot AuCl_3$ (Betainechloroaurate)		43.14% Au

The chloroplatinate, easily soluble in water, formed orange yellow prisms and melted at 246°C.

0.2322 g. subst.	0.0699 g. Pt	30.10% Pt
Calc. for $(C_5H_{11}NO_2 \cdot HCl)_2PtCl_4$ (Betainechloroplatinate)		30.25% Pt

(b) Dissolved portion by absolute alcohol: Saturated alcoholic solution of $HgCl_2$ was added to this portion.

The hydrochloride of the base obtained from the $HgCl_2$-precipitate, formed colourless, hygroscopic, and large prisms and gave the alloxan reaction. Yield: 4.20 g. as hydrochloride. The chloroaurate formed yellow mossy crystals, and was sparingly soluble in water; the melting point was determined as 259°C.

0.1671 g. subst.	0.0739 g. Au	44.23% Au
0.1716 g. subst.	0.0757 g. Au	44.11% Au
0.1628 g. subst.	0.0724 g. Au	44.47% Au
Calc. for $C_5H_{14}NOCl \cdot AuCl_3$ (Cholinechloroaurate)		44.49% Au

Experiment II.

In the second experiment on the isolation or organic bases from "di-saké", 26 litres of the sample was employed and treated like the first experiment; and the quantities of substances isolated by the second experiment were as follows

Hypoxanthine (as hydrochloride)	0.70 g.
Agmatine (as picrate)	4.60 g.
Betaine (as hydrochloride)	0.40 g.
Choline (as chloroaurate)	32.00 g.

Summary.

(1) In the above experimental result the nitrogenous compounds isolated from "di-saké" are:

	In the first experiment (Sample 20 litres)	In the second experiment (Sample 26 litres)
Hypoxanthine (as hydrochloride)	0.60 g.	0.70 g.
Agmatine	1.40 g. (as nitrate)	4.60 g. (as picrate)
Betaine (as hydrochloride)	0.80 g.	0.40 g.
Choline	4.20 g. (as hydrochloride)	32.00 g. (as chloroaurate)
Ammonia	4.73 g. (determined)	——

(2) It is the interesting fact that ths "di-saké" contains fairly amount of agmatine, which had never been isolated from any other fermentation products.

Feeding Experiments with Decomposition Products of Proteins. III.

By

Shiro Mayeda

(Received June 12, 1933.)

In the previous communications,(1) the author has shown, from the results of feeding experiments with white rats, that the proteins in diet can be entirely replaced by the biuret-free acid-hydrolytic products of proteins when supplemented with tryptophane.

Continuing the studies on this subject, the author has carried out further experiments with the mixture of purified amino acids of the following composition: glycocoll 2%, *dl*-alanine 13%, *l*-leucine 15%, *l*-proline 4%, *l*-oxyproline 4%, *l*-tyrosine 3%, *l*-phenylalanine 3%, *l*-cystine 3%, *d*-glutamic acid 15%, *dl*-aspartic acid 6%, *dl*-tryptophane 2%, mixture of *l*-histidine-, *d*-arginine- and *l*-lysine hydrochloride 30%. For the preparation of the diet used in the experiment, 15 parts of the above amino acid mixture were

added to 65 p. of starch, 15 p. of butter, 5 p. of McCollum's salt mixture, besides 5 p, of alcoholic extract of yeast. When rats were fed on this diet, they soon lost the appetite and the body weights rapidly decreased until all of them succumbed within 2∼3 weeks, So it is evident that the above amino acid mixture could not substitute the proteins in diet, thus confirming the observations of Abderhalden,(2) U. Suzuki,(3) Mitchell,(4) McClendon(5) and Rose.(6)(7)

When, however, the mono-amino fraction of the acid hydrolysate i.e. the filtrate of the phosphotungstic precipitate, was given together with tryptophane and diamino acids, the animals could grow normally, indicating that a certain indispensable factor for growth, which is just lacking in the above amino acid mixture is present in the mono-amino fraction.

W. C. Rose,(7) who is working on the same line, has also come to the same conclusion that the substance essential for growth is contained in the butyl alcohol-soluble portion i.e. in the mono-amino fraction of casein hydrolysate.

With the purpose of isolating this substance, the present author has now separated the mono-amino fraction of the acid hydrolysate of fish meat proteins into 4 parts i. e. (1) fraction less soluble in water, (2) mono-amino dicarboxylic fraction, (3) fraction soluble in alcohol, (4) mono-amino mono-carboxylic fraction. Each fraction was then tested for its supplementing effect by adding it, to the extent of 1.5%, to the basal diet containing the above amino acid mixture. In this way the last (4) fraction alone i. e. the mono-amino mono-carboxylic fraction was proved to be effective for the growth of animals, so it was further converted into copper salts(8) by boiling with copper carbonate and again separated into 3 parts by treating with water and methyl alcohol successively i. e. (1) the part insoluble in water, (2) insoluble in methyl alcohol, (3) soluble in methyl alcohol. Each fraction was then treated with hydrogen sulphide to remove the copper and tested on rats. The result has shown that the favorable growth is only induced by the addition of the 3rd fraction i. e. the copper salt, soluble in methyl alcohol.

In the next experiment, the mono-amino mono-carboxylic fraction i. e. the 4th fraction of the above mentioned was converted into zinc salt(9) by boiling with zinc carbonate and separated into 3 parts i. e. (1) insoluble in water, (2) insoluble in ethyl alcohol and (3) soluble in ethyl alcohol. Each fraction was freed from zinc by treating with hydrogen sulphide and evaporated to dryness. Feeding experiments with these preparations have proved that the 3rd fraction i. e. the zinc salt, soluble in ethyl alcohol alone is effective.

Taking these facts in consideration, the author has proceeded as follows. The mono-amino mono-carboxylic fraction obtained from the acid hydrolysate of fish meat protein was converted into zinc salts and, after complete drying,

it was pulverized and extracted several times with boiling absolute alcohol. The alcoholic solution was then evaporated in vacuum to dryness and extracted again with cold absolute alcohol. This operation was repeated until there was no insoluble residue left. The zinc salt obtained by evaporating the alcohol was then dissolved in water, decomposed with hydrogen sulphide and converted into copper salt in the usual way. The copper salt thus obtained was now treated with cold methyl alcohol, the methyl alcoholic solution was evaporated, dissolved in water and treated with hydrogen sulphide to remove the copper, and filtered from the copper sulphide. When the filtrate thus obtained was evaporated at a lower temperature, a colorless crystalline substance separated out which was recrystallized from 80% alcohol.

Feeding experiments were carried out by adding these crystals to the basal diet containing the amino acid mixture above mentioned. When the rats were previously fed on the basal diet alone they rapidly lost their body weight, but when supplied with 0.5% of the above crystals they began to recover in weight, though slowly. When the amount was doubled, the growth was more rapid, thus in one experiment the rats gained in weight from 74 and 75 grs. up to 88 and 97 grs., resp. in 26 days. So it is clear that this substance played a remarkable rôle upon the nutrition of rats.

This substance crystallizes in colorless needles. Heated in a capillary, it softens at 208–9°, and melts at about 220° with decomposition. It gives no biuret reaction, but gives a typical bluish violet coloration with ninhydrin. It is also precipitated by mercuric acetate in alkaline medium like many amino acids.

The results of analysis are as follows:

4.433 mg. Subst. 7.365 mg. CO_2 and 3.380 mg. H_2O
4.157 mg. Subst. 0.3900 cc. N (756.5 mm., 25.5°C)

	C	H	N
Found :	45.31%	8.53%	10.41%
Calc. for $C_5H_{11}O_3N$: (oxy-amino-valerianic acid)	45.11%	8.27%	10.53%

Apparently it agrees with the formula of oxy-amino-valerianic acid, but it is not yet sure whether this substance is really a single one, and further investigations are necessary to decide whether or not it is identical with oxyvaline, obtained by Schryver and Buston[9] from oat protein.

The author expresses his sincere thanks to Prof. U. Suzuki for his kind advice and encouragement throughout the work.

(Prof. U. Suzuki's Laboratory, The Institute for Physical and Chemical Research.)

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Untersuchungen über die Enzyme von Bombyx mori, L.

III. Mitteilung. Über die Tyrosinase und Katalase des Blutes der Seidenraupen.

Von

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(Eingegangen am 24, Juli 1933)

Eingehende Angaben über die Tyrosinase und Katalase bei Insekten sind sehr selten. In Fortsetzung der Untersuchungen über die Enzyme bei den Seidenraupen,[1][2] teilt nun der Verfasser die Ergebnisse seine Forschungen über diese beiden Enzyme des Blutes derselben mit.

1. Über die Tyrosinase.

(A) *Einrichtung der Versuche*:— Die für meine Versuche verwendeten Seidenraupen wurden im Frühling 1933 unter gleichen Bedingungen auf gezogen. Die quantitative Bestimmung der Tyrosinase geschah der zahlreichen, vergleichenden Untersuchungen wegen durch die einfache, etwas modifizierte BACHsche Methode. Zu diesem Zweck wurden 15 ccm 0.03 proz. l-Tyrosinlösung, 3 ccm M/3 Phosphatpufferlösung von pH 6.64 (mit Ausnahme von Versuch 1.), 1.5 ccm Wasser und 0.5 ccm mit 0.85 proz. NaCl-Lösung dreifach verdünntes Blut vermischt und 30 Minuten lang bei 30° (mit Ausnahme von Versuch 2.) gehalten. Dann wurde die Reaktion durch 2 ccm 10 proz. Schwefelsäure sistiert and mit 0.01 N $KMnO_4$-Lösung bis zur Entfärbung titriert.

(B) *Optimale pH*:— Versuch 1. China-7-B, 2. Tag im V. Lebensalter der Raupe.

pH	6.47	6.64	6 81	7.17	7.38	7.73	8.04
0.01 *N* $KMnO_4$. ccm	3.8	4.4	4.0	3.8	3.4	3.2	2.8

(C) *Optimaltemperatur*:— Versuch 2. China-7-B, 4. Tag im V. Lebensalter der Raupe.

Temp.°	23	30	37	45	55	65
0.01 *N* $KMnO_4$. ccm	1.2	4.1	5.4	4.8	2.8	0.6

(D) *Geschlechtsunterschied*:— Versuch 3. China-7-D, 4. Tag im V. Lebensalter der Raupen.

	♀				♂			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
0.01 *N* $KMnO_4$. ccm	2 8	2.8	3 2	2 9	4 4	3 8	4 0	4 1

(E) *Unterchied zwischen gut gewachsenen und schlecht gewachsenen Raupen*:— Versuch 4. Japan-110-E, 4. Tag im V. Lebensalter.

	Gut gewachsene Raupen.				Schlecht gewachsene Raupen.			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
0.01 *N* $KMnO_4$. ccm	3.8	3 8	4 4	4.0	3 8	4 4	3 6	3 9

(F) *Aenderungen durch Hungerzustand bei den Raupen*:— Versuch 5. Europa-7-A, 4. Tag im V. Lebensalter.

Hungerstunden	1	6	24	50
0.01 *N* $KMnO_4$. ccm	1.2	2 4	1.6	0.8

(G) *Unterschied zwischen den verschiedenen Rassen der Seidenraupen*:— Versuch 6. V. Lebensalter. 0.01 *N* $KMnO_4$ ccm.

Rasse / Tage	Japan-110-E	Japan-110-G	China-7-B	China-7-D	Europa-7-A	Europa-7-C
2	6.4	6.0	—	3.4	2.8	0.8
4	4 0	3.4	6.2	3.2	1.2	0.8
7	4.4	4.0	10.6	4.8	2.2	2.4

(H) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori, L.*:— Versuch 7. Europa-7-A.

	Raupe	Einspinnen des Kokons		Puppe		
Tage	Reife	2	4	1	3	5
0.01 *N* $KMnO_4$ ccm	4.2	0.6	0.2	0.4	0.6	0.4

	Puppe			Schmetterling	
Tage	8	11	13	1	3
0.01 *N* $KMnO_4$ ccm	0.3	0.4	0.4	5.6	6.2

II. Über die Katalase.

(A) *Einrichtung der Versuche*:— Als Substrat benutzte ich der gleich zeitig durchgeführten, zahlreichen Versuche und ferner der kräftigen Katalasewirkung des Bluts wegen etwas konzentrierte, namlich ca. 0.3 Proz. H_2O_2-Lösung. 25 ccm H_2O_2-Lösung, 3 ccm M/3 Phosphatpufferlösung von pH 6.64 (mit Ausnahme von Versuch 1.), 1.5 ccm Wasser und 0.5 ccm mit 0.85 proz. NaCl-Lösung dreifach verdünntes Blut wurden gemischt und nach 30 Minuten bei 30° (mit Ausnahme von Versuch 2.) in Kölbchen mit 10 proz. Schwefelsäure eingelassen. Dann wurde die nicht zersetzte Menge Wasserstoffsuperoxyd duch Titration mit einer 0.01 *N* $KMnO_4$-Losung bestimmt.

(B) *Optimale pH*:— Versuch 1. China-7-B, 3. Tag im V. Lebensalter der Raupe.

pH	6.47	6.64	6.81	7.17	7.38	7.73	8.04
Gespaltene H_2O_2 mg	18.70	20.94	20.20	19.45	17.95	16.64	14.59
Spaltung %	24.21	26.81	26.14	25.18	23.74	21.30	18.88

(C) *Optimaltemperatur*:— Versuch 2. China-7-B, 4. Tag im V. Lebensalter der Raupe.

Temp °	5	15	23	30	37	45
Gespaltene H_2O_2 mg	11.05	14.28	15.13	11.56	8.84	7.17
Spaltung %	14.30	18.49	19.59	14.96	11.44	9.24

(D) *Geschlechtsunterschied*:— Versuch 3. China-7-D, 4. Tag im V. Lebensalter der Raupen.

	♀				♂			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
Gespaltene H_2O_2 mg	14.21	13.09	13.46	13.59	17.95	17.58	18.70	18.08
Spaltung %	18.40	16.82	17.43	17.55	23.74	22.75	24.21	23.57

(E) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen*: Versuch 4. China-7-D, 7. Tag im V. Lebensalter.

	Gut gewachsene Raupen				Schlecht gewachsene Raupen			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
Gespaltene H_2O_2 mg	16.08	15.71	15.33	15.77	13.46	12.72	12.34	12.84
Spaltung %	20.82	20.33	19.75	20.30	17.43	16.46	15.97	16.62

(F) *Änderungen durch Hungerzustand bei den Raupen*:— Versuch 5. Europa-7-C, 4. Tag im V. Lebensalter.

Hungerstunden	1	6	24	50
Gespaltene H_2O_2 mg	7.48	7.85	6.73	8.23
Spaltung %	9.68	10.17	8.71	10.65

(G) *Unterschied zwischen den verschiedenen Rassen der Seidenraupen*:— Versuch 6. V. Lebensalter.

Tage	2		4		7	
Rasse	Gespaltene H_2O_2 mg	Spaltung %	Gespaltene H_2O_2 mg	Spaltung %	Gespaltene H_2O_2 mg	Spaltung %
Japan-110-E	16.08	20.82	14.96	19.30	15.71	20.33
Japan-110-G	24.31	31.47	20.19	26.14	19.82	25.66
China-7-B	——	——	10.71	13.86	15.47	20.03
China-7-D	19.89	25.75	13.47	17.44	14.59	18.89
Europa-7-A	8.16	10.56	6.68	8.65	7.85	10.16
Europa-7-C	6.29	8.14	7.48	9.68	10.10	13.07

(H) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori, L.*:— Versuch 7. Europa-7-A.

	Raupe	Einspinnen des Kokons		Puppe		
Tage	Reife	2	4	1	3	5
Gespaltene H_2O_2 mg	11.22	21.32	20.94	19.82	40.02	31.56
Spaltung %	14.52	27.60	27.09	25.66	51.81	40.85

	Puppe			Schmetterling	
Tage	8	11	13	1	3
Gespaltene H_2O_2 mg	35.36	14.11	11.97	18.50	25.57
Spaltung %	45.77	18.27	15.50	23.95	33.10

Zusammenfassung.

(1) Die Tyrosinase des Bluts von Bombyx mori wirkt am besten bei pH 6.6. Die Optimaltemperatur liegt bei 37°.

(2) Die Tyrosinasewirkung des Bluts ist bei den Männchen etwas starker als bei den Weibchen, aber es besteht kein Unterschied zwischen den gut gewachsenen und den schlecht gewachsenen Raupen. Durch Hunger wird die Wirkung der Tyrosinase erst etwas starker und dann allmahlich schwächer.

(3) Der Tyrosinasegehalt des Bluts der japanischen und der chinesischen Rassen ist recht viel höher als derjenige der europaischen. Bei den reifen Larven ist die Tyrosinase sehr aktiv, aber mit dem Beginn des Spinnens der Kokons tritt eine rapide Abnahme auf und während der Puppenperioden bleibt sie bedeutend zurück. Dann wieder vermehrt sich die Tyrosinase in den Tagen des Schmetterlingslebens rasch bis zu einem Maximum.

(4) Die Blutkatalase von Bombyx mori zeigt die optimale Aktivität bei pH 6.6; ihr Temperaturoptimum liegt bei 23°.

(5) Die Katalasewirkung des Bluts ist bei den Mannchen etwas stärker als bei den Weibchen und auch bei gut gewachsenen Raupen etwas höher als bei schlecht gewachsenen. Es wurde aber innerhalb der zwei Hungertage keine Änderung beobachtet.

(6) Der Katalasegehalt des Bluts der japanischen und der chinesischen Rassen ist recht viel höher als derjenige der europaischen. Beim Beginn des Kokonspinnens der reifen Larve findet eine rapide Zunahme der Katalaseaktivitat statt, die ihr Maximum meist am dritten Tage der Puppenperiode erreicht. Dann sinkt die Katalase allmählich und wird in der Periode des Schmetterlingslebens wieder bedeutend stärker.

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On the Two Kinds of Saponin of Soya Bean.

By

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(Received Oct. 21, 1933)

We have isolated easily and in large quantity the two kinds of saponin, crystal and amorphous, from the alcoholic extraction of soya bean as the by-product in the process of the alcoholic extraction method of the soya bean oil.[1] The crystalline saponin had been investigated by Y. Sumiki[2] already and he reported that it formed squama, melting at 222~4°, the molecular formula was $C_{49\sim52}H_{70\sim84}O_{21}$, by the hydrolysis sapogenin ($C_{32\sim35}H_{48\sim56}O_3$), glucose, rhamnose, arabinose and other unknow acidic substance were obtained, and its haemolytic and toxic power were very weak. E. Walz[3] had reported that there were three kinds of saponin in soya bean, but those details remained unexplained. We have compared the two saponins and further investigated them closely as the first step toward the utilization of alcoholic extract.

Experimental.

1) Isolation :— Alcoholic extract of soya bean, from which the bean oil was separated and alcohol was evaporated, is mixed with NaCl-solution and from separated precipitate the ether soluble matter is removed, then the residue is recrystallized from 80% alcohol repeatedly and crystal and amorphous ones are separated by fractional crystallization.

2) Molecular formulas and properties :—

(A) Crystalline saponin. mel. pt. 225~227°. Its properties are identical with those of Sumiki and natrium-salt is hexagonal plate, decomposing at 259°, both have not haemolytic power.

analysis ; $C_{48\sim50}H_{77\sim81}O_{18}$, cal. H 8.25~8.43%, C 61.21~61.92%
obs. H% 8.66 8.58 9.00 9.43, C% 61.03 61.04 61.07 61.86
mol. wt. cal. 941~969, obs. 960 964 992

(B) Amorphous saponin. mel. pt. 216~218°. White powder is recry-

stallized from butyl alcohol and its natrium-salt decomposes at 260° and both have not haemolytic power.

analysis ; $C_{45\sim51}H_{79\sim83}O_{19}$, cal. H 8.20~8.37%, C 60.56~61.26%.
obs. H% 8.86 8.83 8.67 8.52, C% 61.24 60.75 60.41 60.70.
mol. wt. cal. 971~999, obs. 1160 1154.

3) The products by the hydrolysis :—

(A) Hydrolysis. Each saponin is heated in 80% alcohol-5% sulfuric acid 5~40 hours and after evaporation of alcohol sapogenin insoluble in water is filtered off and from the water solution sulfuric acid is removed by neutralization with baryta and then bariumsalt of glucuronic acid is obtained by adding alcohol to the concentrated water solution and next from alcohol solution the mixture of monosaccharides is gained as syrupy mass.

(B) Glucuronic acid is so accertained that above obtained bariumsalt is estimated barium content (26.25%) and it forms the *p*-Br-phenyl-osazon-glucuronic acid-barium (mel. pt. 214°) and phenyl-osazon (mel. pt. 204°).

(C) Galactose from the fraction of monosaccharides is established by the formation of music acid (mel. pt. 216°) and methyl-phenyl-hydrazon (mel. pt. 191°). (after treating with diphenylhydrazin to remove the arabinose wich is derived from glucuronic acid).

(D) Rhamnose is proved as *p*-Br-phenylhydrazon (mel. pt. 213°) (after galactose above mentioned is removed) and phenylosazon (mel. pt. 161°). Above three products are identical in both crystal and amorphous saponin.

(E) Sapogenin is obtained five isomers as the period of hydrolysis and studied its mutual relation.

[1] shows the sapogenin from the crystalline saponin.
[2] 〃 〃 〃 〃 〃 amorphous saponin.

(A) Sapogenins soluble. in carbon tetrachloride and ligroin.

(1) Yellow amorphous (hydralysis 5 hrs.)
([1] mel. pt. 75~80~128° [α]=+25.4°
[2] mel. pt. 95~120° [α]=+48.0°)

↓ (hydrolysis in 80%. alcohol-5% H_2SO_4. 10~20 hrs.

(2) Needle crystal (〃)
([1] mel. pt. 235°. [α]=+74.0°
[2] mel. pt. 224~226 [α]=+87.3°)

(hydrolysis above the same ↓ ↑ (1% alcoholic KOH 4 hrs. boiling

(3) Needle crystal (some what short) (hydrolysis 15~40 hrs.)
([1] mel. pt. 239° [α]=+81.7°
[2] mel. pt. 232° [α]=+107.8°)

kalifusion

hydrolysis

(B) Sapogenins insoluble in the same solvent.

(1) Needle crystal (when ever) (soluble in chloroform)
([1] mel. pt. 260° [α]=0
[2] mel. pt. 293~294° [α]=+9.6°)

↑ (1% alcoholic KOH 4 hrs. boiling

(2) Rectangle plate crystal (hydrolysis 40 hrs.) (insoluble in chloroform)
([1] mel. pt. 248~249° [α]=0
[2] mel. pt. 313° [α]=+115.8°

The products of kalifusion and hydrolysis of the (B) sapogenins are almost analogous to yellow amorphous and crystal of (A) sapogenins respectively, but those specific rotation deviates a little from these value.

These all sapogenins are recrystallized from methylalcohol or benzol, and soluble in alcohol, ether, but insoluble in water. They are not lacton and are neutral with the exception of faintly acid of (A) [1] which gives by the titration with alkali the twice of the value of molecular weight as monobasic acid. Moreover it is interesting that if these saponins and sapogenins are heated with copper oxide or lead chromate by means of Dumas method, they liberate the considerable volume of methan or ethan gas.

The analytical data of these sapogenins differ a little each other, therefore they are not true isomer, but we have been able to consider the following formula.

1. $C_{30\sim32}H_{48\sim52}O_3$

		H%			C%			mol. wt.	
cal.		10.53~10.83,			78.95~79.34,			456~484.	
obs.	(A) [1]	10.51	10.88		78.71	78.39		469	473
	[2]	10.81	10.15	10.33	79.51	79.38	79.07	492	465
	[3]	11.13	10.81		79.95	80.11		460	493
	(B) [1]	11.16	10.57	10.72	78.68	79.03	79.07	474	
		10.82	10.70		79.86	79.64			
	[2]	10.51	10.28	10.58	78.91	79.20	79.36	442	501
		10.76			79.58				

2. $C_{31\sim33}H_{50\sim54}O_4$

		H%			C%			mol. wt.
cal.		10.37~10.58,			76.54~77.04,			486~514
obs.	(A) [1]	10.33	10.15		77.21	76.98		512
	[2]	11.02	10.73		76.83	76.62		472
	[3]	10.55	10.22		77.20	77.53		461
	(B) [1]	10.97	10.65	10.70	76.66	76.92	77.05	507
		10.74			76.74			
	[2]	10.51	10.28	10.71	76.35	76.10	76.49	467

Then these formulas are resemble to those of the acidic sapogenins respectively, oleanolic acid $C_{30}H_{48}O_3$ or $C_{31}H_{50}O_3$ and hederagenin $C_{31}H_{50}O_4$. And if we add each one molecule of glucuronic acid, galactose, and rhamnose to this formula, by the following equation it is equal to the formula of each saponin.

[1] $(C_{30\sim32}H_{48\sim52}O_3 + C_6H_{10}O_7 + C_6H_{12}O_6 + C_6H_{12}O_5) - 3H_2O = C_{48\sim50}H_{77\sim81}O_{18}$

[2] $(C_{31\sim33}H_{50\sim54}O_4 +$ 〃 $+$ 〃 $+$ 〃 $) - 3H_2O = C_{49\sim51}H_{79\sim83}O_{19}$

(F) Acetyl derivatives of sapogenin :—

We obtain the following acetyl derivatives from each sapogenin by ordinary method.

[1] Diacetyl sapogenin. mel. pt. 175°. Needle crystal.

$C_{35}H_{54}O_6$. cal. H 9.83% C 75.81%, mol. wt. 554.
obs. H% 10.14 10.24, C% 75.36 75.77, mol. wt. (Rast) 549.
(titration) 226×2=452, ×2.5=565, ×3=678.

[2] Triacetyl sapogenin. mel. pt. 212°, Needle crystal.

$C_{38}H_{58}O_7$, cal. H 9.34%, C 72.84%, mol. wt. 626.
obs. H% 9.80 10.07, C% 73.33 73.67, mol. wt. (Rast) 637.
(titration) 193×3=579, ×3.5=675, ×4=772.

It is not explained why the results of the estimation of molecular weight by the titration deviate from the true value and what is the form of the remaining oxygen which is not carbonyl, but has been able to perform methylation by methyl-sulfate.

Summary.

(1) We have studied the crystalline saponin of soya bean which Y. Sumiki had investigated already, and have obtained the results that its molecular formula is $C_{48\sim50}H_{77\sim81}O_{18}$ and the products of hydrolysis are sapogenin ($C_{30\sim32}H_{48\sim52}O_3$), glucuronic acid, galactose, and rhamnose.

(2) Now we have isolated at the same time an amorphous saponin, of which molecular formula is $C_{49\sim51}H_{79\sim83}O_{19}$. Its sapogenin is $C_{31\sim33}H_{50\sim54}O_4$ and the others are identical with the former. Both have not haemolytic power.

(3) Each saponin is isolated five isomers according to the period of the hydrolysis and is required each mutual relation. And we have showed that the molecular formula of these neutral sapogenins are respectively resemble to those of the acidic sapogenins, oleanolic acid and hederagenin.

(4) If these saponins and sapogenins are heated with copper oxide or lead chromate, they liberate the considerable volume of methan or ethan gas. We have isolated the two more saponins that are water-soluble and haemolytic (crystal melts at 270° and amorphous 243°), of which we are to report later. Then we think that three kinds of saponin E. Walz showed are the following kinds of ours. One of them (mel. pt. 225°) is the crystalline saponin, ones (mel. pt. 280° hexagonal) is its natrium-salt and the remains (mel. pt. 272° haemolytic) are the saponin that is water-soluble.

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Studies on the Germination of Seeds. Part III.

Transformation of Carbohydrates During Germination of Soy-Bean Seeds.

By

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With regard to the carbohydrates of the matured soj-bean seeds, Prof. M. Yukawa[1] made a very valuable investigation and found the following substances :—

Total carbohydrates (in anhydride form)	21.69%		
Cane sugar	5.90%	Galactan	4 64%
Stachyose	3.52%	Fiber (crude)	3.85%
Araban	3.80%		

The present paper is to treat briefly the transformations of the foregoing substances, and also reducing sugar and starch, which appear in the seedlings of soy-beans during germination.

Method of Analysis.

Reducing sugar :— The samples were extracted with hot 90% alcohol, clarified with lead acetate, and estimated directly by Bertrand's method and expressed as glucose.

Cane sugar :— The samples were treated with alcohol and lead acetate as in the case of reducing sugar, and inverted with dilute HCl. Then, the total reducing power was determined. Cane sugar was calculated by subtracting from this value which the reducing sugar and the stachyose theoretically indicate.

Stachyose :— Stachyose was calculated from the difference between the weight of mucic acid obtained from all the specimens and that from the residues extracted with hot 90% alcohol.

Starch and dextrin :— Starch and dextrin were estimated together as they were difficult to separate. The residues extracted with hot 90% alcohol were digested with malt extract, and then the reducing power corresponding to starch and dextrin was determined in the usual manner.

Araban :— Araban was determined by Ôshima and Kondô's furfurol method[2]. Pentosan and methyl-pentose were also estimated by the same

method, but the amounts of these substances were rather insignificant and not worth mentioning.

Galactan :— Galactan was estimated by the mucic acid method adapted by H. D. Dore[3] from the residues extracted with hot 90% alcohol.

Crude fiber :— Crude fiber was determined as usual.

Results of Analysis.

White autumn seeds (larger specimens) of the bean produced in Korea were used as material for the experiment. The seeds, and the seedlings plucked out every 2, 5, 7, and 10 days after sowing in a dark room were analysed. The figures shown in the following table indicate the per cent of the original weights of the soj-bean seeds used :—

Seeds		Seedling (days after sowing)			
		2	5	7	10
Reducing sugar	0.5	2.5	4.7	2.7	1.0
Cane sugar	5.0	3.1	1.9	0.8	0.5
Stachyose	3.7	2.4	0.8	0.5	0.4
Starch and dextrin	0.4	2.0	5.5	7.3	9.0
Araban	4.5	4.9	5.2	5.7	6.1
Galactan	4.9	4.0	3.5	3.1	2.9
Crude fiber	3.6	3.8	4.2	4.8	5.2
Total	22.6	22.7	25.8	24.9	25.1

Summary.

Reducing sugar, which is scarcely detected in the seeds, is increased the earlier stage of germination and decreased afterwards. Cane sugar and stachyose are decreased gradually. Starch and dextrin are markedly increased. Galactan is decreased ; araban and crude fiber are increased. The total amounts of carbohydrates do not show any marked changes, during the development of the seedlings in the periods of the experiment.

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Über die Bildung des Harnstoffs aus Prolysin, Citrullin, verschiedenen Hydantoinen und aus Eiweisskörpern durch Einwirkung von Schwefelwasserstoff in schwach alkalischer Lösung.

Von

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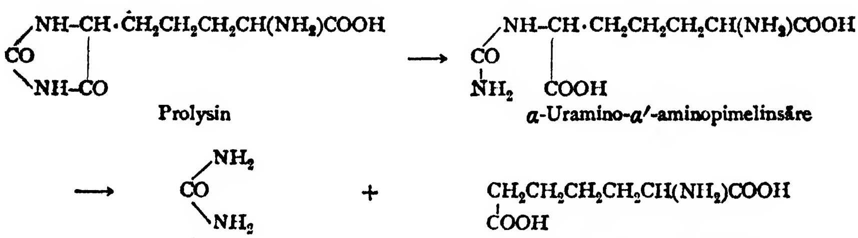
(Eingegangen am 1, November 1933)

In der vorigen Arbeit[1] hat Wada nachgewiesen, dass der Hydantoinring des Prolysins im Gegenwart von Bariumcarbonat durch Einwirkung von Schwefelwasserstoff in Harnstoff abgespalten wird und zwar nach folgendem Schema :—



Prolysin — α-Uramino-α'-aminopimelinsäre

Harnstoff + α-Monoaminopimelinsäure

Spater wurde festgestellt, dass das Hydantoin des Prolysins, d. h. Pentamethylendihydantoin, Citrullin, sowie die Hydantoine der gewöhnlichen Aminosäuren wie Leucin, Phenylalanin usw. in analoger Weise unter Bildung des Harnstoffs gespalten werden.

Neulich ist es den Verfassern gelungen, die Bildung des Harnstoffs unmittelbar aus Eiweisstoff durch Einwirkung von Schwefelwasserstoff nachzuweisen. In diesem Fall wurde aber die Reaktion in verdünnter ammoniakalischer Lösung vorgenommen, um den Eiweisstoff in Lösung zu bringen.

Der gebildete Harnstoff wurde als freie Substanz oder als Nitrat, Oxalat, und Dixanthylderivat gereinigt und identifiziert. Da die Spaltung des Harnstoffs aus Prolysin oder Citrullin durch Schwefelwasserstoff fast quantitativ verläuft, so ist es möglich die Menge des Prolysins und Citrullins im Eiweissmolekül aus dem gebildeten Harnstoff zu ermitteln.

Experimenteller Teil.

1. a) 0,2 g Citrullin und 0,2 g Bariumcarbonat wurden in 20 ccm Wasser verteilt, 10 Minuten auf dem Wasserbad erwärmt und dann Schwefel-

1) Proc., 9 (1933), 43~46; Biochem. Zeitschr., 262 (1933) 57~67.

wasserstoff durchgeleitet. Die Uraminogruppe des Citrullins wird dadurch reduziert und spaltet Harnstoff ab. Wenn das Reaktionsgemisch mit Äther wiederholt extrahiert und der ätherische Extrakt verdampft wide, so erhält man den Harnstoff als Dixanthylverbindung vom Schmelzpunkt 260 bis 261°. Der oben erwähnte ätherische Extrakt gibt die P. Ehrlichsche sowie die Schiffsche Reaktion sehr stark. Durch Einwirkung von Urease in wässeriger Lösung wird Ammoniak gebildet.

b) 2 g Leucinhydantoin wurden in 50 ccm verd. Ammoniak (0.08 n) gelöst, 15 minuten auf dem Wasserbad erwärmt und in oben erwähnter Weise behandelt. In diesem Fall entsteht nebst Harnstoff Isobutylessigsäure, welche in wasser schwer lösliches, blaues Kupfersalz bildet. Im Kapillarrohr erhitzt, zersetzt sich das Kupfersalz bei 293°.

Analyse des Kupfersalzes von Isobutylessigsäure:

1,962 mg Subst.,	0,529 mg CuO,	Cu=21,27%.
Ber. für $(C_6H_{12}O_2)_2Cu$,		Cu=21,65%.

Zur quantitativen Bestimmung des gebildeten Harnstoffs wurden 0,0100 g Leucinhydantoin in 50 ccm einer mit Magnesiumoxyd gesättigten Lösung gelöst und Schwefelwasserstoff durchgeleitet. Man setzte nun soviel Essigsäure zu bis die Lösung gegen Phenolrot neutral reagierte, und nach dem Vertreiben des Schwefelwasserstoffs wurde die Lösung mit 1 ccm 10%iger Urease Lösung und wenig flüssigem Paraffin versetzt, und 15 Minuten auf dem Wasserbad bei 50° erwärmt um den gebildeten Harnstoff in Ammoniak zu verwandeln. Nach dem Erkalten wurde das gebildete Ammoniak mit Schwefelsäure in bekannter Weise titriert.

H_2SO_4 verbraucht: 1,4 ccm(1 ccm=0,004561 g H_2SO_4)=0,0063854 g H_2SO_4.
=39,0% Harnstoff.
Ber. für Leucinhydantoin =38,4% Harnstoff.

Man sieht, dass die Spaltung des Harnstoffs in diesem Fall fast quantitativ verlaufen ist.

c) 5 g Phenylalaninhydantoin wurden in 100 ccm verd. Ammoniak gelöst und genau in analoger Weise behandelt wie Leucinhydantoin. In diesem Fall bildet sich an Stelle der Isobutylessigsäure Phenylpropionsäure. Das Kupfersalz der letzteren kristallisiert glanzenden hellgrünlich blauen Nadeln. Es schmilzt bei 215° unter Zersetzung.

Analyse des Kupfersalzes von Phenylpropionsäure:

2,100 mg Subst.,	0,465 mg CuO,	Cu=17,69%.
Ber. für $(C_9H_9O_2)_2Cu$		Cu=17,57%.

2. Bildung des Harnstoffs aus Casein durch Einwirkung von Schwelwasserstoff in ammoniakalischer Lösung.

100 g Casein wurden in 200 ccm verd. Ammoniak (0.08 n) gelöst und

nach dem Zusatz von 800 ccm einer mit Magnesiumoxyd gesättigten Lösung 20 Minuten auf dem Wasserbad erwärmt und Schwefelwasserstoff durchgeleitet. Um den gebildeten Harnstoff von Eiweiss zu befreien, wurde die Lösung mit Essigsäure angesäuert, abfiltriert und eingedampft. Der dadurch zurückgebliebene Rückstand wurde mit Äther extrahiert. Beim Verdampfen des Äthers schied sich der Harnstoff als farblose Nadeln aus. Der letztere ist in Wasser und Alkohol löslich, schmilzt bei 132° und gibt die P. Ehrlichsche sowie die Schiffsche Reaktion sehr stark. Es wird durch Einwirkung von Urease in wässeriger Lösung in Ammoniak gespalten. Mit Xanthydrol bildet er Dixanthylharnstoff vom Schmelzpunkt 260 bis 261°. Die Ausbeute an Harnstoff betrug 1.8% des Caseins.

Analyse des Harnstoff:

2,104 mg Subst.,	0,855 ccm N_2(758,5 mm 24°),	N=46,54%.
Ber. für N_2H_4CO,		N=46,66%.

Analyse des Dixanthylharnstoffs:

5,364 mg Subst.,	0,303 ccm N_2(757,5 mm 24°),	N=6,47%.
Ber. für $N_2H_2CO \cdot 2(C_{13}H_9O)$,		N=6,66%.

Die Bestimmung des aus verschiedenen Eiweisskörpern gebildeten Hanstoffs wurde in analoger Weise ausgeführt wie bei Leucinhydantoin. In diesem Fall wurde aber die in Lösung gehaltenen Eiweisstoffe durch Einleiten des Schwefelwasserstoffs teilweise wieder gefällt und die Reaktion verlief nicht glatt, so dass ein viel niedrigerer Wert erhalten wurde als man erwartete. Im folgenden werden Resultate tabellarish zusammengestellt:

	gelöst in NH_4OH+MgO		Verbraucht H_2SO_4	= Harnstoff
(1) Edestin ....2,0535 g	50 ccm	30 ccm	17,9 ccm	2,43%
2,0225	"	"	17,6	2,43
(2) Zein 1,0123	30	"	1,9	0,52
0,9140	"	"	1,6	0,48
(3) Ovalbumin 2,0490	"	50	1,3	0,18
2,0138	"	"	1,2	0,16
(4) Glycinin ....0,7029 (extrahiert mit 0.2% NH_4OH)	50	20	1,9	0,75
(5) Oryzanin..................1,0246 (extrahiert mit 0.2% NH_4OH)	"	"	1,7	0,46
(6) Casein...... 2,0296	"	"	13,6	1,88
2,0153	"	"	13,6	1,89
(7) Casein...................2,4251	"	—	12,6	1,45
2,4342 (extrahiert mit 0.2% NaOH)	"	—	14,0	1,51
(8) Gelatine..................3,0808	—	50	21,1	1,91
(9) Fibrin 0,9176	50	"	6,0	1,82

Da Zein kein Prolysin in seinem Molekül hat, so muss der Harnstoff ausschlizsslich aus Citrullin gebildet werden.

A Study on the Effect of Fatty Acids on Nutrition.

II.—Experiments with Diets composed of Rice, Oil and Lipoid containing Linoleic or Linolenic Acid.

By

Ume TANGE.

(Received August 23, 1933.)

When young rats were restricted to fat-free diets, they developed characteristic symptoms accompanying impairment of growth, denuded areas on skin and "scaly" condition of feet, and they were cured by the administration of either linoleic or linolenic acid[1]. This observation led the author to further determination as to what function oils and phospholipins containing these essential acids play in animal physiology.

Recently Evans and Lepkovsky[2] indicated that the fatty acids from rice-starch were very potent in relieving disease in rats fed on a fat-free diet, while the fatty materials from potato-starch were ineffective. In our experiments, however, when potato-starch in the fat-free diet was replaced by either polished-rice powder or rice-starch, the growth of the animals was arrested and the fur was stained with blood, which was supposed to be bled around the nose and mouth. In this case, unfortunately, the sick animals were not cured with either linoleic or linolenic acid even though a high level of the yeast extract was administered, but the substitution of whole dried yeast resulted in a marked growth, recovering the ill conditions. This evidence suggested, therefore, the possibility of the presence in the yeast of other growth factors, not present in the yeast extract.[3][4]

Experimental.

Preparation of Materials used in the Experiments.

a) Lecithin :— Purification of lecithin from "Soyalex".*

Two hundred grams of "Soyalex" were extracted with ether at room temperatur. Into the extract two volumes of pure acetone were added, and allowed to stand until a precipitate had settled out. This precipitated the phospholipins and left most of the fat and cholesterol in solution. The clear solution was then decanted from the precipitate, redissolved in ether and

* "Soyalex" (the crude lecithin prepared from soy-bean by the alcohol extraction method in the Central Institute of the South Manchurian Railway Company) was kindly furnished to the author by the Ohzeki Company in Tokyo.

reprecipitated, and this was repeated until the ethereal solution was entirely clear. By the addition now of three volumes of absolute alcohol to each volume of ether, nearly all of the cephalin was precipitated, but lecithin remained in solution. Into the filtrate a warm solution of $CdCl_2$ in 85% alcohol was added until no more precipitate formed. The lecithin-$CdCl_2$ precipitated out as white crystals. This was washed several times with absolute alcohol until the filtrate had become colourless. The lecithin-$CdCl_2$ was now suspended in 95% alcohol and decomposed with alcoholic saturated solution of $(NH_4)_2CO_3$. The filtrate was then evaporated under reduced pressure in CO_2 atmosphere, and the residue was treated with ether to remove the admixture of $(NH_4)_3CO_3$ and Cd salts. By the addition of acetone into the ether extract the lecithin precipitated out as a pale-yellow waxy substance. This was washed several times with acetone and dried in vacuum. About 40 g of lecithin were obtained, which had an iodine value of 75 (Wijs). The analysis of total N (Kjeldahl): 2.00% and P (molybdate-method): 4.31%, N/P: 1.08/1.00.

Separation of saturated and unsaturated fatty acids in lecithin.

Forty grams of lecithin obtained by the method mentioned above were heated on a water bath for 2～3 hours with saturated $Ba(OH)_2$ solution, and the resulted Ba soaps were now decomposed with 10% HCl. By extracting the hydrolyzed liquid with ether, about 20 g of fatty acids were obtained, which had an iodine value of 108 (Wijs). The mixed acids were converted into lead salts by the usual method; the lead salts of the unsaturated fatty acids were removed by thorough extraction with ether. After separating the ether-soluble lead salts from the insoluble ones, each of them was hydrolyzed in ether medium with HCl. Thus, 5.0 g of saturated acids having m p 56～57°C and 7.5 g of unsaturated acids having an iodine value of 130 (Wijs) were obtained.

b) Preparation of vitamin B_2:— The most potent concentrate of vitamin B_2 recorded in the literature seems to be that obtained by Narayanan and Drummond[5] by means of adsorption by fuller's earth from yeast extract of pH 0.1.

Two hundred grams of the baker's yeast powder were treated in like manner as described in a previous paper,[1] except with alcohol of 50% concentration. After extracting the concentrated yeast extract with ether to remove fat completely, a saturated solution of $Pb(C_2H_3O_2)_2$ was added into it. The lead acetate precipitate was decomposed by suspending it in warm water and slowly adding 10% H_2SO_4 with constant stirring until acid to congo-red, whereupon the lead sulphate was removed by filtration. In order to remove any trace of lead that might be present, H_2S was passed through the filtrate

to saturation and the whole allowed to stand for several hours. The reaction of the filtrate was then rendered approximately 0.9 N with H_2SO_4, being almost at pH 0.1, and treated with 3 g of fuller's earth for every 100 c.c of the filtrate.

The mixture was well stirred mechanically for 1/2 hour, then filtered and the earth was well washed with 0.9 N H_2SO_4. The filtrate and washing were similarly treated, a second time with 1.5 g of fuller's earth for each 100 c.c.

Thus, 35 g of the "activated" fuller's earth were obtained, adsorbing nearly 2.5% of the original yeast, and 0.5 g of the earth corresponded to 0.0715 g of adsorbed organic matter.

c) Neutral fatty oil from soy-bean oil :— This was made by dissolving soy-bean oil in a mixture of ether and alcohol (1 : 1) and by treating it with about 5% in excess of the amount of alcoholic KOH needed to neutralize the free acids in the oil. The mixture was occasionally shaken and allowed to stand for some hours at room temperature, then it was diluted with distilled water and extracted several times with ether. The ethereal solution was washed with distilled water until the filtrate showed no more alkaline reaction. After dehydrating with anhydrous Na_2SO_4, the solution was evaporated as completely as possible in a high vacuum in CO_2 atmosphere.

The neutral oil thus obtained consisted of some palmitin and stearin, most of the liquid fatty glycerides of soy bean oil and also a very little amount of unsaponifiable substances. The saponification and iodine values are as follows :—

Oil	Acid value	Saponif value	Iodine value
Original soy-bean oil	0 479	193	139 (Wijs)
Neutral fatty oil	——	196	139 (Wijs)

d) Saponification of cod-liver oil :— Fifty grams of cod-liver oil were introduced under constant stirring into 125 c.c of 20% methyl alcoholic KOH solution and left overnight at room temperature, then the hydrolyzed product was added into the alcoholic solution containing the calculated amount of $CaCl_2$ in order to convert the K-soap into Ca-soap. The precipitate was decomposed with dilute H_2SO_4, and the separated fatty acids were extracted with ether. The subsequent procedure was the same as described in the part of the neutral fatty oil from soy-bean oil. Thus a pale yellowish semi-solid substance having an indine value of 129 was obtained.

e) Rice-bran and chrysalis oils.†

Oil	Saponif. value	Iodine value
Rice-bran oil	185	107 (Wijs)
Chrysalis oil	194	115 (Wijs)

Tsujimoto[6] stated that fatty acids of rice-bran oil consisted of 20% palmitic acid, 45% oleic acid and 35% isolinoleic acid.

The analytical data of chrysalis oil by Kimura[7] are listed in the following Table:

Table I.

Acid value	Sap. value	Iod. value	Rhodan value	Unsap. matter %	Total fat %			
1.57	191.58	141.8	93.0	0.98	93.08			
					Saturat. acids %	Unsaturated acids %		
						Oleic acid	Linoleic acid	Linolenic acid
					23.9	22.2	27.3	26.6

Feeding of Animals.

Male albino rats of 40∼50 g were kept two or three in a cage with the various diets listed in Table II. All the diets were supplemented with the known necessary factors as follows: Vitamin A, D and B were supplied by administering biosterol*, irradiated ergosterol* and alcoholic extract of yeast or whole dried yeast respectively. Semi-solid oils were given by dissolving them in liquid paraffin; otherwise, the technique of feeding was the same as in the previous experiments.[1]

Table II. Composition (in g) of diets.

Diet	I (Fat-free diet)	II	III	IV	V	VI	VII	VIII	IX
Casein (fat-free)	20	20	20	18	18	18	18	20	20
Potato-starch (fat-free)	76							75	
McCollum salt mixture[8]	4	4	4	4	4	4	4	4	4
Polished-rice powder		76		75	75				75
Rice-starch			76						
Rice-bran				3					
Whole dried yeast					3				
Half-polished rice, retaining about 40% germ.						78			
Unpolished rice							78		
Lecithin								1	1

† I wish to thank Mr. Y. Kawakami of the Kao-Soap Company for funishing the oils.

* I wish to thank Dr. M. Sumi and Dr. J. Nakamiya for supplying the irradiated ergosterol and biosterol.

Results and Discussion.

a) The growth of rats receiving the polished rice (II) or the rice-starch diet (III) was very much inferior to those receiving the potato-starch diet (I). To our surprise the growth on the two former diets was retarded from the beginning, and the fur and the fore paws were mostly stained with red pigments like blood, and the abnormal condition of fur, giving an appearance of cotton, was often noticed (one of examples being illustrated by Photo.).

Following the bareness around the nose, the mouth and the eyes, there was a tendency to lose the hair on the whole body. The bald areas looked moist and inflamed, but showed no definite characteristic symptoms developed with the fat-free diet, namely the scurfy condition of the skin, "scaliness" of the feet and etc.

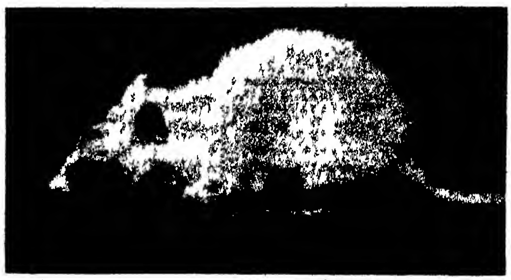


Photo.—Showing the onset of a characteristic symptom of a rat which had been fed with the polished-rice diet (Diet II).

The sick animals on these rice diets could not be recovered in spite of the increased use of the yeast extract, or cured by linoleic acid* unless the rice in the diets was replaced by potato-starch or the yeast extract by whole dried yeast. This fact called our attention to the possibility of the presence of toxic substance or substances in rice, which may be neutralized by whole dried yeast, because the substitution of it for the yeast extract brought about a marked improvement, showing resumption of weight and soft fine hair on the denuded areas.

It was an outstanding phenomenon that rats receiving the polished-rice diet in which 3% of rice-bran (IV) were added or those receiving the half-polished-rice diet (VI) developed the symptom resembling that on the polished-rice diet (II), though in the case with the half-polished-rice diet (VI) the rats showed very satisfactory growth (Charts 1～3). The rats receiving the unpolished-rice diet (VII) attained a normal growth and appearance (Chart 4).

On autopsy of the rats fed with the rice diets, some lesion was often noticed in the liver and the kidney, sometimes exhibiting a white kidney.

b) Linoleic acid with vitamin B_2:— An attempt was made to determine whether linoleic acid could spare vitamin B_1, as a parallel experiment on vitamin B_2.(1) For this purpose the "activated" fuller's earth described previously in this paper was used as vitamin B_2 source.

* I desire to thank Dr. Y. Sahashi for generously supplying pure linoleic acid.

When fed with ration containing 0.5% of the "activated" fuller's earth, there appeared decline of weight as well as anorexia, and some died in spite of increase of the dose. Since the failure of growth and death were found due to an inadequate technique for the preparation of vitamin B_2, an autoclaved yeast extract[9] was employed instead of the "activated" fuller's earth; then there was a satisfactory growth for some intervals, but gradually the growth was impaired without any onset of the characteristic convulsion of vitamin B_1 deficiency. In this case, the administration of 2 drops daily of active oryzanin* induced a remarkable growth on the rats (Chart 5).

This evidence, therefore, led to conclude that linoleic acid had no "sparing" action on vitamin B_1 as in the case with B_2.[9]

c) Lecithin:— The rats reared on the diet containing 1% lecithin manifested an optimum growth; the fur was very fine and lustrous. In 1932, Trautman[10] stated that the feeding of 2 g daily of lecithin to a guinea pig accelerated growth, while 4 g retarded it. In this experiment, when given in the level of 1.5% of lecithin, growth was definitely improved but a proportion higher than 1.5% was not used since the pure lecithin was insufficient to ascertain Trautmann's experiment (Chart 6).

d) Neutral fatty oil of soy-bean oil:— Drummond and Gregory[9] have reported that the cause of the failure of the rats on the dietaries containing synthetic fat was due to a toxic substance, which was probably produced during the prolonged heating necessary for the esterification. This suggestion led the author to use the natural fatty oil free from fatty acids.

When 3 drops daily of the fatty oil were given the rats grew as healthy as those with 2 drops daily of linoleic acid itself. The oil had an effectiveness in alleviating the deficiency disease produced by the fat-free diet when the symptoms were not too advanced. It was, however, found that its curing power was less than that of linoleic acid itself (Charts 7 and 10).

e) Fatty acids of cod-liver oil:— As growth had been unsatisfactory on the diet containing 0.5% of the fatty acids, 2 drops daily of 30% of the acids dissolved in liquid paraffin were given, which exerted no appreciable influence on growth, and the animals had very similar symptoms described on the fat-free diet.[1] When a larger dose was given there was a tendency to produce diarrhea (Chart 8).

f) Chrysalis oil:— The group fed with 2 drops daily of the oil showed an excellent growth with a fine lustrous coating of hair. When the weight had exceeded 200 g the oil was replaced by 2 drops of 30% rice-bran oil dissolved in liquid paraffin, but the animals could continue growth until the

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* I am indebted to Dr. S. Odake for supplying rhe active oryzanin which cured severe antineuritic pigeons with 4 mg daily.

experiment was terminated (Chart 9 A).

g) Rice-bran oil :— The feeding was started with the diet containing 1% rice-bran oil, but the animals soon lost their appetite and began to decline the weight, and there appeared the loss of hair around the nose and eyes. Though administered *per os* 2 drops daily of 30% oil dissolved in liquid paraffin or of chrysalis oil, no appreciable influence was exhibited either on growth or on malnutrition. When this condition continued, the animals have become emaciated and been unable to be relieved even though linoleic acid was administered, with the exception of one rat (Chart 9 B).

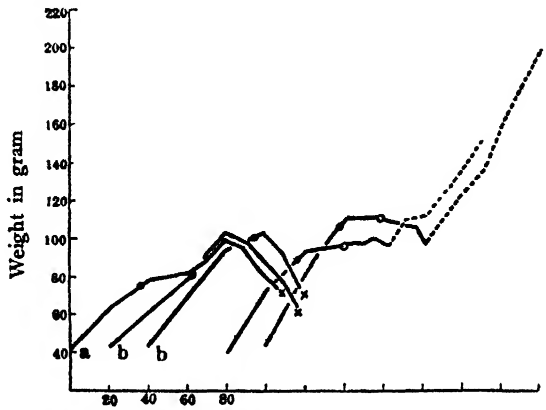
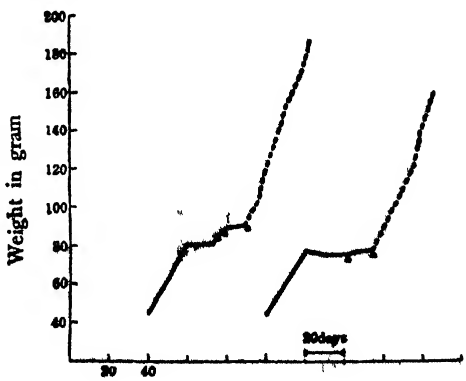


Chart 1.—Curves showing the growth of rats on Diet II before and after the substitution of whole dried yeast for the yeast extract, with administration of 2 drops daily of linoleic acid (a) or without the acid (b). The dotted line indicates the replacement of the yeast extract by whole dried yeast (Diet V), representing the prompt recovering of symptoms and resuming of weight. The small spot indicates an occurrence of bleeding and the small circle shows the sign ef losing hair on the body; × indicates death.

Chart 2.—Curves showing the growth of rats on Diet IV without administration of linoleic acid. During the period △—△ the yeast extract was furnished, and thereafter 3% whole dried yeast replaced it and at the same time the rice-bran was withdrawn. The small spot indicates an occurrence of bleeding.



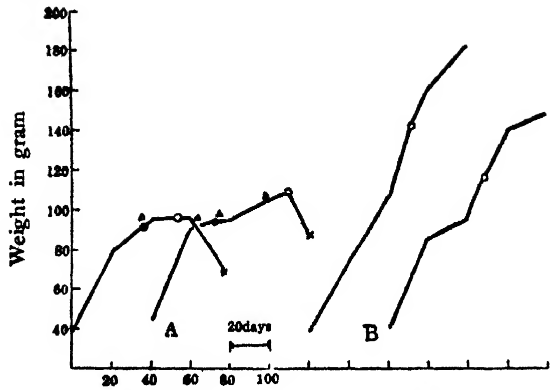


Chart 3.—Curves A showing the growth of rats on Diet III, supplemented with the yeast extract, but without linoleic acid. During the period △—△ a larger level of the yeast extract was employed, but showed no appreciable influence on growth. The small spot indicates an occurrence of bleeding and the small circle shows sign of bareness on the body; × indicates death.

Curves B showing the growth of rats on Diet VI without linoleic acid as well as the yeast extract. The small circle indicates an occurrence of losing hair on the body.

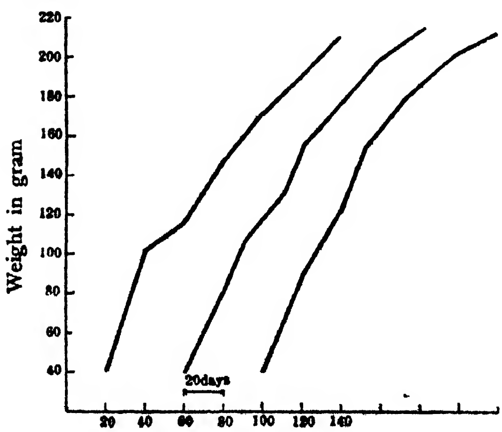


Chart 4.—Curves showing the growth of rats on Diet VII, without linoleic acid as well as the yeast extract.

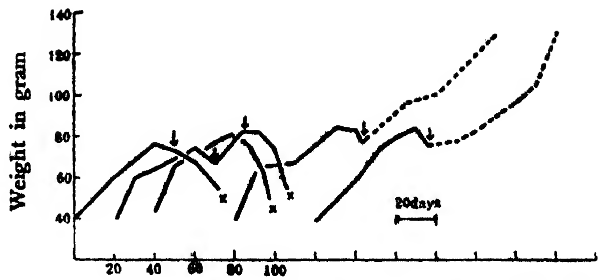


Chart 5.—Curves showing the growth of rats on Diet I, supplemented with "activated" fuller's earth or autoclaved yeast extract as vitamin B_2 source, and the renewed growth by supplying 2 drops daily of oryzanin solution as vitamin B_1. The arrow indicates adding of oryzanin solution; × indicates death.

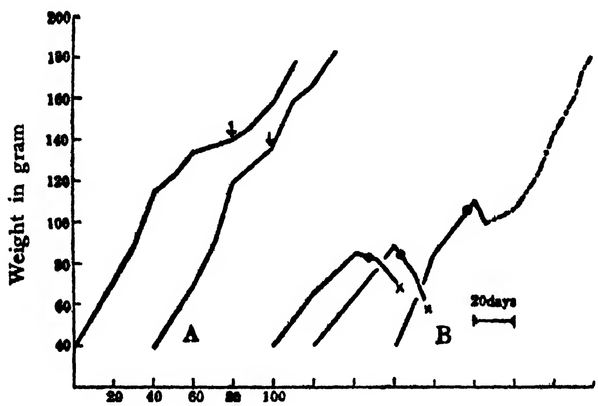


Chart 6.—Curves A showing the growth of rats on Diet VIII. The arrow indscates increase of lecithin to the level of 1.5%.

Curves B showing the growth of rats on Diet IX, and with the replacement of Diet VIII there appears the renewed growth and recovery from symytoms. The small spot indicates an occurrence of bleeding; × indicates death.

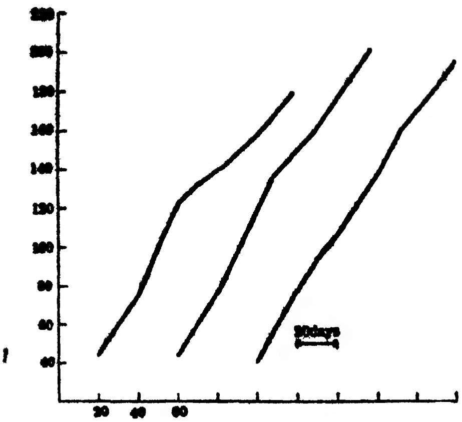


Chart 7.—Curves showing the growth of rats on Diet I, administered with 3 drops daily of neutral fatty oil of soy-bean oil.

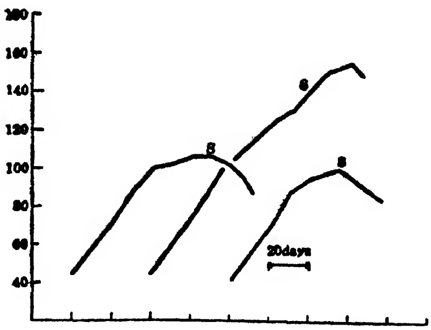


Chart 8.—Curves showing the growth of rats on Diet I, supplemented with 2 drops daily of fatty acids of cod-liver oil; s indicates "scaly" feet condition.

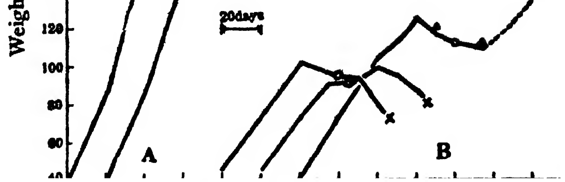


Chart 9.—Curves A showing the growth of rats on Diet I, administered with 2 drops daily of chrysalis oil. The broken line indicates the substitution of rice-bran oil for chrysalis oil.

Curves B showing the growth of rats Diet I, supplemented daily with 2 drops of rice-bran oil. During the period △—△ the oil was replaced by chrysalis oil. The dotted line shows the renewed growth and recovery from symptoms with administration daily of 2 drops of linoleic acid. The small circle indicates an occurrence of losing hair on the body; × indicates death.

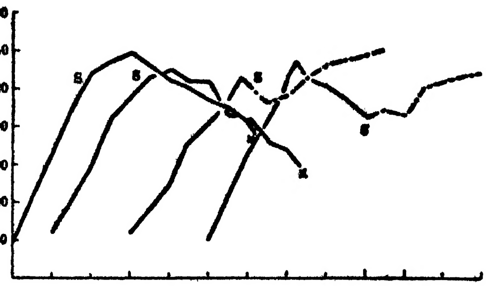


Chart 10.—Curves showing the growth of rats on Diet I, and renewed growth and slow recovery from the deficiency disease by treatment with 3 drops daily of fatty oil of soy-bean oil; s indicates "scaly" feet condition; × indicates death.

Summary.

(1) Growth and the general condition of animals are not attained by the diets containing 76∼78% of polished-rice powder, rice-starch, or half-polished rice as carbohydrates, except unpolished-rice diet, though an adequate amount of alcoholic yeast extract is provided. When, however, the yeast extract is replaced by whole dried yeast there appears a prompt recovery of the abnormal condition. The most characteristic phenomena are the occurrence of bareness on the body and of bleeding around the nose, the mouth, and the fore paws.

(2) Lecithin and soy-bean oil possess an almost equal nutritive value in animal physiology to linoleic acid.

(3) Chrysalis oil induces an excellent growth in rats, owing to its content of unsaturated fatty acids, namely linoleic and linolenic acids.

(4) The abnormal condition in nutrition of the rats fed on the rice-bran oil is not improved by administration of either chrysalis oil or linoleic acid. The cause of malnutrition and of the failure of growth with the former oil is not yet clear.

(5) The results of experiments with fatty acids prepared from cod-liver oil show that they cannot maintain the growth of animals and that the large dose causes a disturbance of the gastro-intestine.

(6) Linoleic acid is unable to "spare" either vitamin B_1 or B_2.

The author wishes to express her deep gratitude to Professor U. Suzuki for his many helpful suggestions throughout this work. She is indebted to the Keimei Society for financial assistance and is also grateful to Miss K. Miti for her willing help in feeding of the animals and preparing of the diets.

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